HAPPY BIRTHDAY ENRICO!

A WELCOME PRESENCE SOUTH OF GREEN STREET!

THE SUPERHIGHWAY BETWEEN UIUC BIOCHEMISTRY AND PHYSICS
THE EDUCATIONAL LEGACY ... GREGORIO -> ENRICO ->

THE FIRST “LFD”
SLIGAR LABORATORY AND THE LFD

IN 1972 I WAS SUPPOSED TO WORK ON THE RED-EDGE EFFECT!

Enrico: Recall discussions on University Avenue over a soldering iron?
... MANY TOOLS, MANY DISCOVERIES

LATER, IT WAS ALSO ALL ABOUT PRESSURE

c.1982: 390 RAL, ACROSS THE HALL FROM GREGORIO
IT'S REALLY ALL ABOUT LEARNING

FOUR EXAMPLES:

• MEMBRANES AS A SINGLE MOLECULE
• BLOOD COAGULATION
• RECEPTORS AND SIGNALING
• CELL MIGRATION / SEEING FORCE
SELF-ASSEMBLED NANOMETER SCALE LIPID BILAYERS FOR ELUCIDATING THE STRUCTURE AND FUNCTION OF INTEGRAL MEMBRANE PROTEINS

PROTEINS AND LIPIDS AT THE SINGLE MOLECULE LEVEL: RECEPTORS, ARRAYS, IMAGING

ADVANCING SANCHEZ & GRATTON
MEMBRANE PROTEINS OFFER UNIQUE CHALLENGES TO BIOCHEMISTRY AND STRUCTURAL BIOLOGY

**BILAYER ASSOCIATED SYSTEMS**
- VIRAL INFECTION / SENSING
- G-PROTEIN COUPLED RECEPTORS
- CHANNELS AND TRASPORTERS
- DRUG METABOLIC ENZYMES
- HEMOSTATIC ENZYMES

**PROBLEM:**
ISOATING THESE SYSTEMS IN A FUNCTIONAL, SOLUBLE STATE

Normal isolation yields:

UOVA STRAPAZZATE
WHAT WE DISCOVERED:
A SIMPLE SELF-ASSEMBLY PROCESS TO SOLUBILIZE MEMBRANE PROTEINS WITH NATURALISTIC PRESENTATION AND NATIVE ACTIVITY

TARGET

MEMBRANE SCAFFOLD PROTEIN

+ PHOSPHOLIPIDS

SOLUBLE AND MONODISPERSE AT THE SINGLE MOLECULE LEVEL

QUEST:
USE NANODISCS FOR DRUG DISCOVERY, DIAGNOSIS AND THERAPEUTIC DELIVERY

THE NANODISC®
DESIGN & EXPRESSION OF MEMBRANE SCAFFOLD PROTEINS

TOTAL GENE SYNTHESIS

(NH₂)-MGHHHHHHIEGR-HIS₆-F-X

GT[spacer]-(COOH)

6-HIS TAG X HELICAL SCAFFOLD PROTEIN REPEATS

600 mg/L !!!

E. coli CODONS
MINIMAL RNA 2° STRUCTURE
RESTRICTION SITES

BL21(DE3)

0 1 2 3 6 19 MSP MW

t (h) →

3 2/3 RESIDUES / TURN AMPHIPATHIC HELIX
MAKING A GRAM OF MUTANT GLOBINS FOR HANS AND COLLEAGUES!
... THEN MYOGLOBIN BECAME HEMOGLOBIN!

5 gm / L  E. coli

BUT I DIGRESS .....
PRECISION SELF-ASSEMBLY OF 10 nm NANODISCs

NANODISC MEMBRANE BILAYERS ARE ROBUST AND IMMUNOLOGICALLY NEUTRAL IN HUMAN

5 ns MOLECULAR DYNAMICS

MSP1T2
160 DPPC
9.7 nm Dia.

COMPATIBLE WITH MANY CHEMICAL & BIOPHYSICAL PLATFORMS

- STOPPED FLOW
- LYOPHILIZE / RE-SOLUBILIZE
- CELL SORTER / MICROFLUIDICS
- SURFACE IMMOBILIZATION

BIGGER BELT - BIGGER NANODISC

SOLUTION X-RAY SCATTERING

12.1 nm
10.9 nm
9.7 nm
8.5 nm

Q, Å⁻¹

ILIA DENISOV AT APS DND-CAT

Denisov et al. (2004) JACS 126, 3477-3487
NANODISC PHOSPHOLIPIDS HAVE NORMAL BILAYER PROPERTIES

- SCANNING CALORIMETRY
- X-RAY SCATTERING
- LAURDAN FLUORESCENCE
- DPH POLARIZATION

Denisov et al. (2004) JACS 126, 3477-3487
Denisov et al. (2005) J. Phys. Chem. 109, 15580
LFD TO THE RESCUE! SINGLE MOLECULE NANOMEMBRANES

- SIZE EXCLUSION CHROMATOGRAPHY
- X-RAY / LIGHT SCATTERING
- ELECTRON MICROSCOPY
- SINGLE PARTICLE SPECTROSCOPY

TWO PHOTON CONFOCAL FCS

\[ D = 20 \, \mu^2 \, s^{-1} \]
WILD HYPOTHESIS: INTEGRAL MEMBRANE PROTEINS COULD BE SELF-ASSEMBLED INTO NANODISC VIA THE SAME PROCEDURE

7-TM RECEPTORS, P450s, CHANNELS, INTEGRINS, TISSUE FACTOR etc.

NH₂

MEMBRANE SCAFFOLD PROTEIN MSP-1

HIS

COOH

TARGET

+ DETERGENT

DIALYZE

IT WORKS!

+ PL

XTAL

NMR

ASSAY

SENSOR
MEMBRANE ELECTROSTATICS AND FUNCTION:
CONTROL OF MANY PHYSIOLOGICAL PROCESSES
BLOOD COAGULATION

HOW TO UNDERSTAND MOLECULAR RECOGNITION OF PROTEINS AND LIPIDS AT THE SINGLE MOLECULE LEVEL
“SLAVING” BY A MEMBRANE

Nanoscale activator of blood clotting
Factor VIIa
Tissue factor
Nanoscale membrane bilayer
PROTEASES IN THE COAGULATION CASCADE

RECRUITMENT TO A MEMBRANE SURFACE

NORMAL ARTERY

PLAQUE

THROMBOSIS

IX

IXa

TF:VIIa

VIIa

X

Xa

TF:VIIa

PT

Thrombin

Activated Platelets

FIBRIN CLOT
COAGULATION FACTOR BINDING

HOW TO DECONVOLUTE THE ENSEMBLE AVERAGE? FCS & PCH!

FVII  10 GLA
FX    11 GLAÇ
THE TISSUE FACTOR - NANODISC COMPLEX:

TF - NANODISCS - BOTH
IN VITRO AND IN VIVO
CONTROL OF HEMOSTASIS

VASCULAR DAMAGE

CURRENTLY:
TESTING
IN SWINE
CARDIOVASCULAR
BLEEDING
MODEL.
ANTIANGIOGENESIS?

HEMOSTASIS

ENRICO:
IRVINE
DOESN’T
HAVE A
PIG FARM!
NEXT STORY: SLIGAR LAB Responds to the Call of Circe …

Proteins that wedge themselves into the fatty membranes surrounding cells are among the most important molecules in modern medicine. But as the cells’ gatekeepers, they detect key compounds outside the cell and determine which should be allowed inside. Just one class of these proteins, G protein-coupled receptors (GPCRs), is a target for drugs that gross sales top $39 billion a year. Studying such gatekeepers in detail is extremely difficult because removing them from the cell membrane almost invari-ably alters their shape and reduces their function.

“You take these guys out of their normal environment, and you get scrambled eggs,” says Stephen Sligar, a physicist and chemist at the University of Illinois, Urbana-Champaign. But now Sligar’s team has found a way to put them on display while keeping it simple as if they’ve never left home.

At the meeting, Sligar reported that he and his colleagues created tiny lipid-based membranes, sus- pended individual GPCRs inside them, and tracked the pro- teins as they executed their chemical signals across the fatty membrane. “It’s a very cool technique,” says Robert Hamers, a chemist at the University of Wisconsin, Madison. “You can see all kinds of applications for something like this.”

Hamers says nanoflakes could shed light on the biochemical behavior of a host of membrane proteins that have escaped detailed understandings. In time the method may also make it possible to crystallize membrane proteins to obtain atomic-level maps of their structure using X-ray crystallography, another long-elusive goal.

Like native cell membranes, the discs are composed of two layers of phospholipid molecules, each sporting water-friendly head groups and long, oily, water-repellent tails. In watery environs, phospholipids curl up into two-layered sheets, with the oily tails in both layers facing inward and the relatively water-friendly head groups pointing out into the water. In cells, the sheets curl into a sphere to form the cell’s protein-coated membrane. Hydrophobic membrane proteins, such as GPCRs, dis- solve in these layers to avoid contact with water. For decades researchers have tried to study membrane proteins in artificial mem- branes, such as spherical lipid structures called liposomes. Unfortunately, it’s hard to see inside the bubble.

To keep the membranes from forming spheres, Sligar’s group had to protect lipid groups along the edges of the membrane from water. Two years ago the researchers succeeded by engineering pro- teins that linked together to form a ring that was water-friendly on the outside and lip-id-friendly on the inside. When they combined this engineered protein with the phospholipids, the two formed tiny nanoscale protein discs, each filled with a tiny phospho- pholipid membrane. Since then, Sligar’s team has discovered a num- ber of different membrane proteins inside the discs.

For their current experiment, Sligar and colleagues teamed up with researchers at 3-Dimensional Pharmaceuticals in Exton, Pennsylvania, who supplied them with copies of a GPCR called the β2 adrenergic receptor (β2AR), a well-characterized protein that is the target of heart drugs called beta-blockers. Using their ring-protein tech- nique, Sligar’s graduate student Andrew Late created nanoflakes resembling disk cells, each wrapped around a single copy of β2AR. The researchers then removed the discs with a small dropletlike compound that in cells binds to the portion of the receptor facing out of the cell. The binding triggers β2AR to change shape and release a G protein, which in cells binds to the inside of the receptor. Using radioactive compounds, the group found that the receptors in the discs traced the same key steps. The result opens the door to studying dozens of less-understood membrane proteins.

That’s not all. Sligar has also teamed up with X-ray crystallographers to try to create crystals in which millions of copies of a membrane protein are all oriented precisely the same way. Crystals of water-soluble pro- teins are used to create atomic maps of dozens of different proteins every year, leading to new drugs and treatments for countless disorders. Sadly, membrane proteins have been all but impossible to crystallize, but Hamers says the nanoflakes should give researchers a way not only to keep them stable but also to chirvify them into alignment. Sligar’s team hasn’t made nanoflake crystals yet. But Sligar says: “We’re working very hard on it.”
G-PROTEIN COUPLED RECEPTOR PHARMACEUTICALS

OVER HALF MARKETED DRUGS TARGET A FEW GPCRs: > $50 B/ yr.

<table>
<thead>
<tr>
<th>RECEPTOR</th>
<th>DISEASE</th>
<th>DRUG</th>
<th>ANNUAL SALES ($ M)</th>
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<tr>
<td>HISTAMINE H2</td>
<td>ULCER</td>
<td>ZANTAC</td>
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<td>ALLERGY</td>
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<td>ANGINA, HYPERTENSION</td>
<td>PROCARDIA</td>
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<td>GABA CHLORIDE RECEPTOR</td>
<td>ANXIETY</td>
<td>XANAX</td>
<td>320</td>
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</table>
BACTERIAL CHEMOTACTIC SIGNALING PATHWAYS

A MODEL FOR OLIQOMEROMIC MEMBRANE PROTEIN SIGNALING
THE BACTERIAL CHEMOTAXIS SIGNALING COMPLEX

ADLER, 1969
RECEPTOR ACTIVITIES AS A FUNCTION OF OLIGOMERIZATION STATE

![Graph showing receptor activities as a function of oligomerization state. The graph plots the percentage of receptors modified against Tar dimers per nanodisc. The x-axis represents the number of Tar dimers per nanodisc, ranging from 0 to 18. The y-axis represents the percentage of receptors modified, ranging from 0% to 80%. There are three curves on the graph labeled 'deamidation', 'methylation', and 'kinase activation'. Each curve is represented by different markers: squares for deamidation, dots for methylation, and triangles for kinase activation. The graph also includes a vertical axis labeled 'Kinase Activation (% Native Vesicle Tar)', ranging from 0 to 40%.](image-url)
OLIGOMERIC STATE CONTROL OF RECEPTOR ACTIVITY

ISOLATED RECEPTOR HOMODIMERS ARE ACTIVE IN:

- LIGAND BINDING
- ADAPTATION MODIFICATION
- TRANSMEMBRANE SIGNALING

TRIMER OF DIMERS REQUIRED FOR:

- KINASE ACTIVATION

UNDERSTANDING THIS REQUIRES FCS AND PCH!

FLUORESCENT LABELS ON RECEPTORS AND NANODISC
UNDERSTANDING CELL MIGRATION

ENRICO: NO PAPERS TOGETHER (YET) - BUT A GRANT!
VISUALIZING INTERNAL FORCES IN THE LIVING CELL

CHANGE THE STRENGTH OF THE FORCE SENSOR BY VARYING THE NUMBER OF COILED-COILS

INSERT FORCE SENSOR BETWEEN REPEATS OF FILAMIN A - CROSSLINKS F-ACTIN FORMING BRANCH NETWORKS

Dimerization Domain
* in 3T3 cells; identical conditions, back to back experiments, same day/time.
WORK IN PROGRESS:

FLIM

Soluble tension sensor:

CHOB7 on FBG (20µg/mL)

FnA-TS II:

FnA-TS null:
CALIBRATING THE FORCE SENSOR

Optical Tweezers

FRET

Anti-Digoxenin Coated Bead

Digoxenin

DNA

MC

PKPQQFM

Transglutaminase Recognition Sequence

Biotin-cadaverin

Streptavidin

MC PKPQQFM
SINGLE MOLECULE GPCR ASSAY, ARRAYS, SENSING, IMAGING

FLUORESCENT LABELING & MICROFLUIDIC MIXING

SINGLE MOLECULE RECEPTOR FRET FCS / PCH

OPTICAL SENSING

DIP PEN NANOLITHOGRAPHY
SO, ENRICO:
WHY LEAVE US FOR CALIFORNIA?!?

FREE SPEECH AREA

THIS AREA HAS BEEN SET ASIDE FOR NON-PROFIT GROUPS TO EXERCISE THEIR CONSTITUTIONAL 1st AMENDMENT FREE SPEECH RIGHTS. THE CITY OF MONTEREY DOES NOT ENCOURAGE OR DISCOURAGE THIS SELLING OF PRODUCTS WHICH WOULD BE UNLAWFUL IF DONE BY OTHERS, AND DOES NOT ENDORSE OR OTHERWISE SUPPORT IN ANY WAY THIS PARTICULAR FORM OF PROTECTED EXPRESSION. COMPLAINTS MAY BE ADDRESSED TO THE MONTEREY CITY ATTORNEY’S OFFICE, 646-3915.
WELL ENRICO …

…YOU TOOK QUITE A BIT WITH YOU …

BUT LEFT BEHIND FAITHFUL COLLEAGUES!
ENRICO: GOOD LUCK AND REMEMBER - “GOD” WILL BE WATCHING!