Structural Genomics of the Human GPCR Protein Family

Julius Axelrod Symposium
ASPET - April, 2010

Stevens Laboratory
The Scripps Research Institute
THIS SLIDE IS UNAVAILABLE.
G Protein-Coupled Receptors

Dozens of Effectors
Thousands of Different Human Receptors (largest family in human genome)
Signaling Mechanism/Specificity (20 years later)
Thousands of Ligands - Chemical Diversity

Our Focus
Signaling Mechanism
Molecular Recognition
Structure-Function

Metabolic enzymes
Ion channels, transporters
Gene expression
Secretory machinery
Stabilized pure GPCR protein for ligand screening – Immediate need and impact

- Stabilized and functional receptor
  - Stabilized in agonist, antagonist, or inverse agonist state
  - Tool compound design for specific conformational states
  - Ability to discover allosteric and dimer binding sites
  - 5 orthogonal assays for novel ligand discovery using purified protein and “ODD” GPCR fragment chemical library
    - GPCR Thermal Stability Analysis
    - GPCR mass spectrometry based size exclusion chromatography for compound library mixture screening
    - Fragment based screen via LCP-CPM assay
    - SPR based kinetic binding assay
    - LCP off-rate assay

Collaboration with NIH Small Molecule Screening Center MLPCN
2.4 Å Structure of Human â² Adrenergic Receptor

Influence of Cholesterol on Receptor Structure and Function – an Allosteric Binding Site (Structure 16, 897, 2008)
Collaborations to understand receptor dynamics in response to different pharmacological compounds

1D NMR spectra of $\alpha_2$AR/DDM in presence of different ligands (Wüthrich Laboratory, TSRI La Jolla)

HDX dynamic mapping of $\alpha_2$ adrenergic receptor (Griffith Laboratory, TSRI Florida)

*Analytical Chemistry*, January 2010

- Apo-form
- Clenbuterol
- Carazolol
- Alprenolol
- ICI118511

% D

- 0-10
- 10-20
- 20-30
- 30-40
- 40-50
- 50-60
- 60-70
- 70-80
- 80-90
- 90-100

- 30 s
- 300 s
- 15 hr
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Distribution and Effects of Adenosine Receptors

Effects:
- CNS modulation
- Cardiac Protection
- Coronary Vasodilation
- Cardiac arrhythmias
- Wound healing
- Anti-inflammatory
- Pain control
- Diuretics

$A_1$:
- Brain
- Heart
- Kidneys
- Lung

$A_{2A}$:
- Brain
- Lungs
- Heart

$A_{2B}$:
- Colon
- Bladder

$A_3$:
- Kidneys
- Liver
- Heart
Outreach - Community-wide Assessment of GPCR Modeling and Docking (GPCR Dock 2008)

(Nature Reviews Drug Discovery June 2009)

A$_{2A}$-ZM241385 Complex

Goal: assess the current state of GPCR modeling and docking methods; similar to CASP and CAPRI

Experiment: registered modelers were given one month to predict and submit up to 10 models of the A$_{2A}$-ZM complex

206 models were received from 29 groups (63 groups registered)

2 Different GPCR Assessment Criteria


- Receptor RMSD
- Receptor-ligand interactions - important for understanding basic molecular interactions as well as structure based drug discovery
  - Ligand RMSD
  - Receptor-ligand contacts
Superposition of All Ligands


- Large variety of binding poses, but clustered around similar location

(crystal structure is shown in blue; model 7msp is shown as spheres; all other models are shown as sticks)
Human Adenosine A2a Receptor at 2.6 Å Resolution

Science (Oct 2nd, 2008)
Phenyl Aromatic ring w. N

Furan

Residues with 4 A radius from ZM241385
Applications of Structure Based Screening

Screen for AA2A R antagonists

- AA2A R crystal structure
- Ligand-guided optimization
- VLS of 4M vendors compounds
- 56 candidates selected
- Binding and functional assays

Applications of Ligand-Guided Modeling (3)

Screen for AA$_{2A}$R antagonists

- AA$_{2A}$R crystal structure
- Ligand-guided optimization
- VLS of 4M vendors compounds
- 56 candidates selected
- Binding and functional assays

- >40% hit rate in experimental binding assays, including 11 submicromolar antagonists
- Novel, chemically diverse (9 chem scaffolds)
- MW<400, high ligand efficiency -> lead like?
- Subtype selectivity vs. A3, but not A1

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**G Protein-Coupled Receptors**

**Martin Rodbell**
1960-1970’s

**Al Gilman**
1970-1980’s

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**Adrenergic Receptor**

**Activated α subunit**

**Inactive G-Protein Activated βγ complex**

**Extracellular Space**

**Adrenaline**

**G Protein**

**Normal leukemia cell**

**Mutated leukemia cell**

**Mutated cell with purified G-protein**

**G-protein extract from brain or other tissue**

**GTP**

**GDP**

**Adrenaline**

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**Discriminator/Receptor Transducer/G-protein Amplifier**

**Cell Interior**

**Cell membrane that effectively separates outside from inside**

**Intracellular Signal – Second Messenger**

**Extracellular Signal – First Messenger**

**Signal A**

**Signal B**

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**Activated α subunit**

**Inactive G-Protein Activated βγ complex**

**Extracellular Space**

**Adrenaline**
G Protein-Coupled Receptors

Activated Adrenergic Receptor

Desensitized Adrenergic Receptor

GrpK phosphorylates activated receptor at multiple sites

Arrestin binds to phosphorylated receptor

Martin Rodbell 1960-1970’s

Al Gilman 1970-1980’s
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