A BRIEF HISTORY OF G-PROTEIN COUPLED RECEPTORS

Nobel Lecture

Stockholm University

December 8, 2012

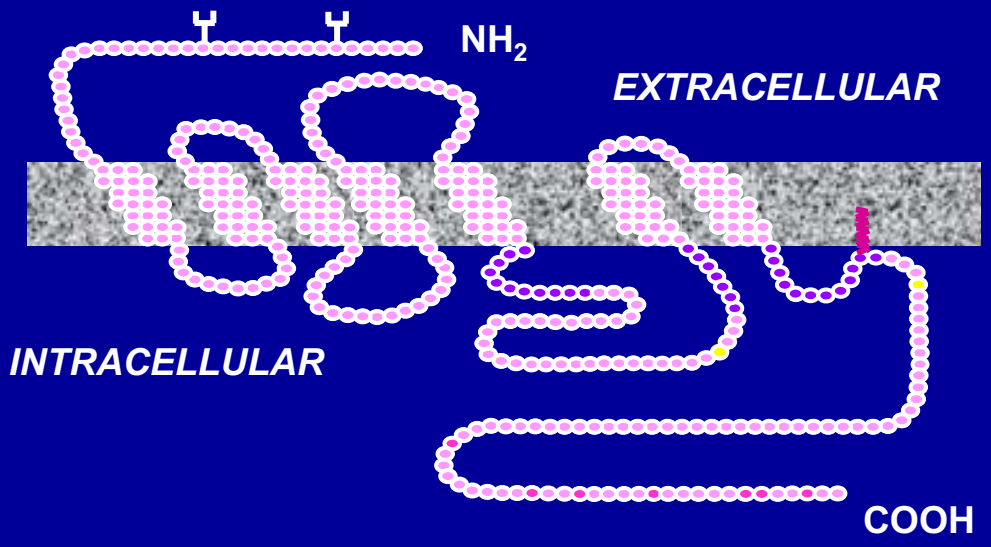
Robert J. Lefkowitz, M.D.

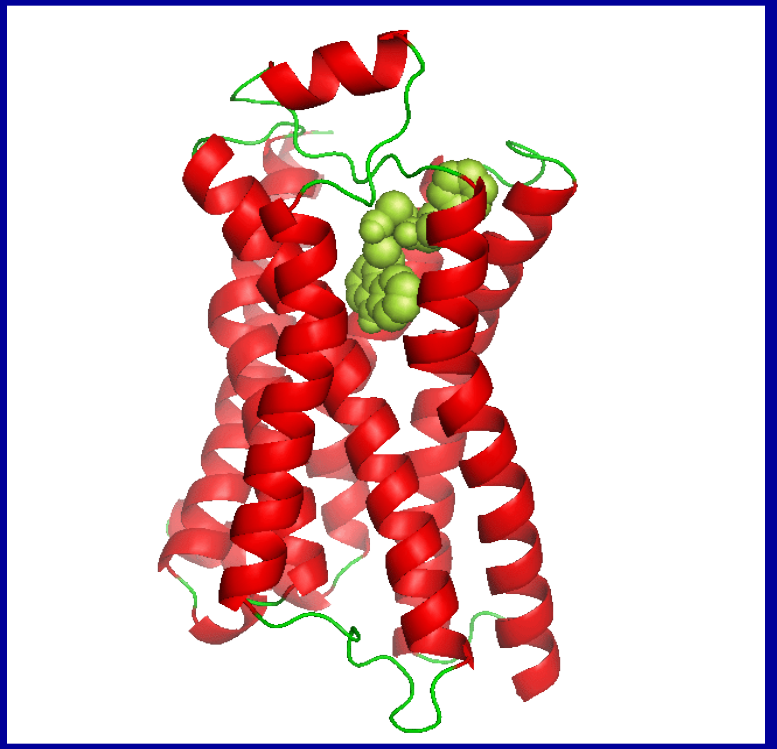
James B. Duke Professor of Medicine

Investigator, Howard Hughes Medical Institute

Duke University Medical Center

G-Protein Coupled Receptors (GPCRs)
Seven Transmembrane Receptors





- ~ 200 functionally known receptors
- ~ 600 functionally unassigned receptors (orphan)
- Hundreds of sensory (taste and smell) and hormone receptors
- Account for about 60% of all prescription drugs
- Examples: α and β-Adrenergic Receptor Blockers and Agonists,
  Serotonin Receptor Blockers and Agonists,
  Histamine Receptor H1 and H2 Blockers,
  Opioid Receptor Blockers and Agonists

A Brief History of Receptors

1900 – 1910 Early Ideas

J.N. Langley (1852-1926)

a) studied the actions of adrenaline and antagonistic drug pairs (nicotine, curare) – skeletal muscle (pilocarpine, atropine) – submandibular gland

b) "receptive substance"

"So we may suppose that in all cells two constituents at least are to be distinguished, a chief substance, which is concerned with the chief function of the cell as contraction and secretion, and receptive substances which are acted upon by chemical bodies and in certain cases by nervous stimuli. The receptive substance affects or is capable of affecting the metabolism of the chief substance" (Journal of Physiology 33, 374-413, 1905)

A Brief History of Receptors

Early Skepticism

H.H. Dale (1875-1968)

"It is a mere statement of fact to say that the action of adrenaline picks out certain such effector-cells and leaves others unaffected; it is a simple deduction that the affected cells have a special affinity of some kind for adrenaline; but I doubt whether the attribution to such cells of "adrenaline-receptors" does more than re-state this deduction in another form." (Transactions of the Faraday Society 39, 319-322, 1943)

A Brief History of Receptors

Later Skepticism

1973 R. Ahlquist *"...This would be true if I were so presumptuous as to believe that α and β receptors really did exist. There are those that think so and even propose to describe their intimate structure. To me they are an abstract concept conceived to explain observed responses of tissues produced by chemicals of various structure"*

(Perspect. Biol. Med. 17:119-122, 1973)

1970-Present The Molecular Era

1970's **Radioligand Binding** ➲ Receptor Regulation

➲ Theories of receptor action

guanine nucleotide effects,

high & low affinity states

➲ Receptor subtypes

Vol. 60, No. 2, 1974 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

STEREOSPECIFIC [^{3}H](-)-ALPRENOLOL BINDING SITES, β-ADRENERGIC RECEPTORS AND ADENYLATE CYCLASE

Robert J. Lefkowitz, Chhabirani Mukherjee, Michael Coverstone and Marc G. Caron

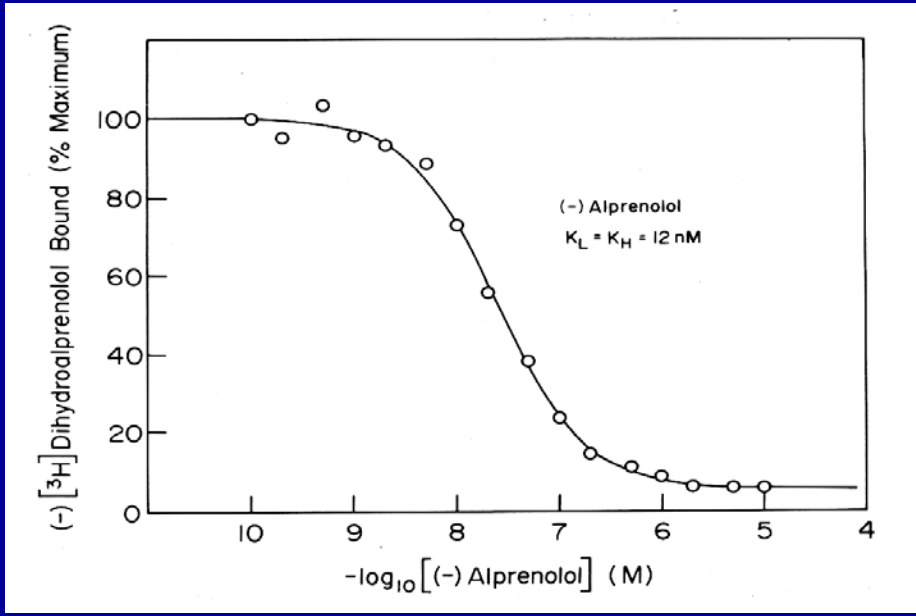
Division of Cardiology, Department of Medicine and Department of Biochemistry
Duke University Medical Center, Durham, North Carolina 27710

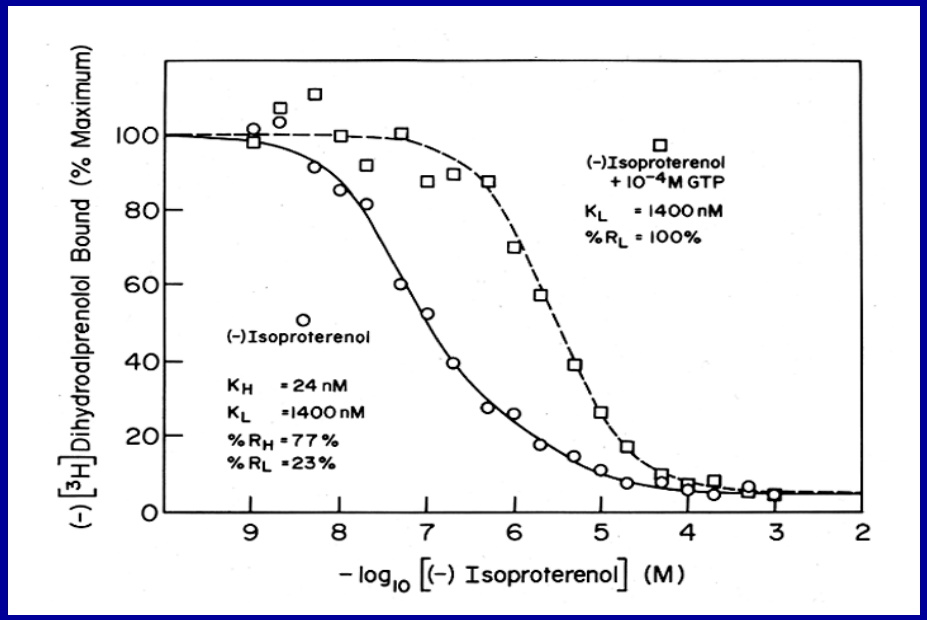
Alpha-Adrenergic Receptor Identification by [^{3}H]Dihydroergocryptine Binding

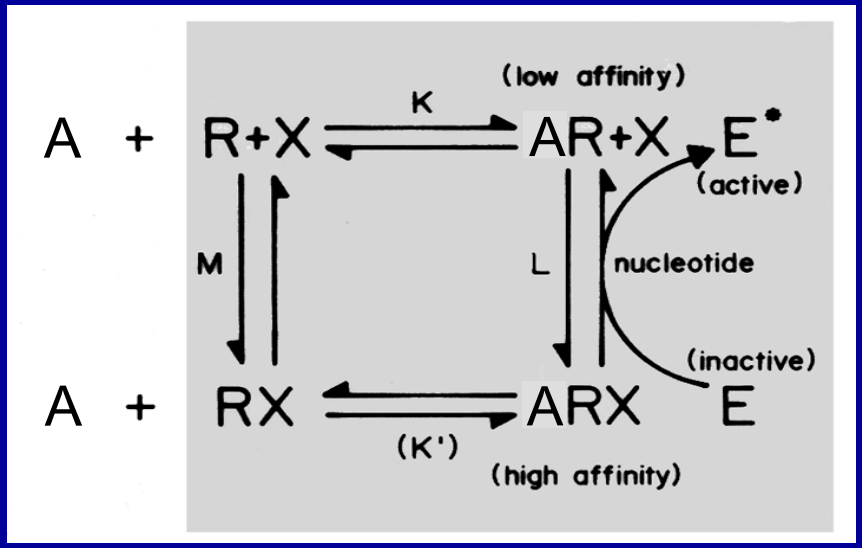
LEWIS T. WILLIAMS ROBERT J. LEFKOWITZ

21 MAY 1976 791 SCIENCE, VOL. 192

Allosteric Regulation of Receptors by G Proteins







THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 255, No. 15, Issue of August 10, pp. 7108–7117, 1980
Printed in U.S.A.

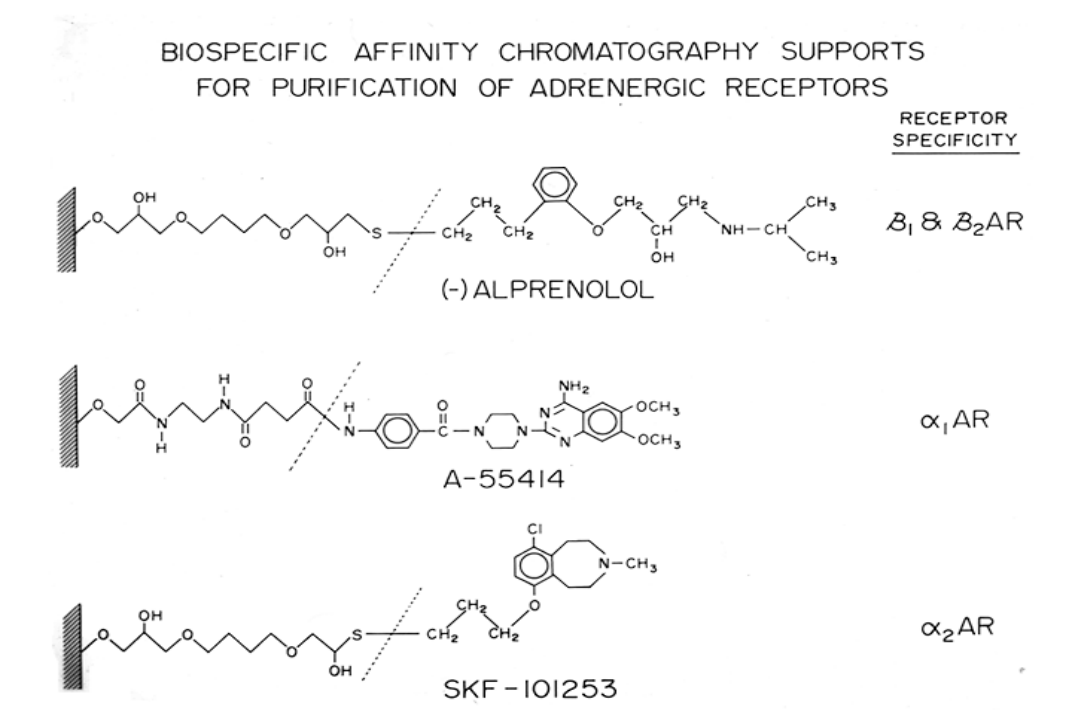
A Ternary Complex Model Explains the Agonist-specific Binding Properties of the Adenylate Cyclase-coupled β-Adrenergic Receptor*

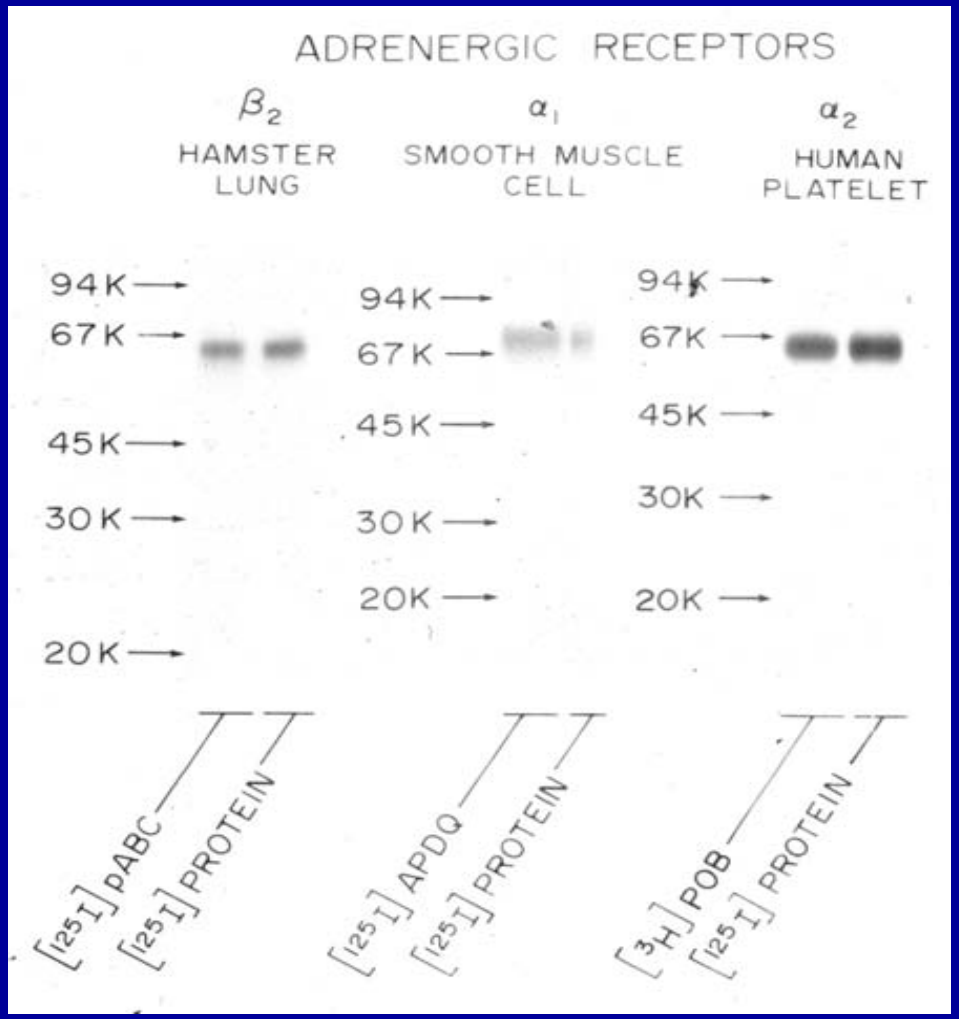
(Received for publication, November 14, 1979, and in revised form, March 18, 1980)

Andre De Lean,‡§ Jeffrey M. Stadel, and Robert J. Lefkowitz¶

From the Howard Hughes Medical Institute Laboratory, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Isolation of Adrenergic Receptors





Receptor Reconstitution

Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 4899–4903, August 1983
Biochemistry

Reconstitution of β-adrenergic receptors in lipid vesicles: Affinity chromatography-purified receptors confer catecholamine responsiveness on a heterologous adenylate cyclase system

(octyl glucoside/Sepharose-alprenolol)

RICHARD A. CERIONE*, BERTA STRULOVICI*, JEFFREY L. BENOVIC†, CATHERINE D. STRADER*, MARC G. CARON*†, AND ROBERT J. LEFKOWITZ*‡

Howard Hughes Medical Institute, Departments of *Medicine (Cardiology), ‡Biochemistry, and †Physiology, Duke University Medical Center, Durham, North Carolina 27710

Communicated by Henry A. Lardy, May 2, 1983

Communication

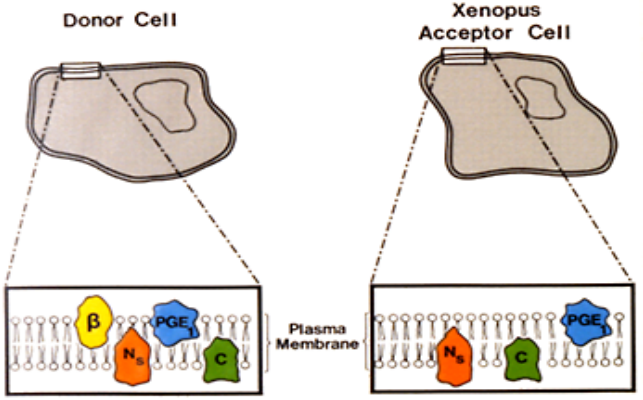
THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 259, No. 16, Issue of August 25, pp. 9979–9982, 1984

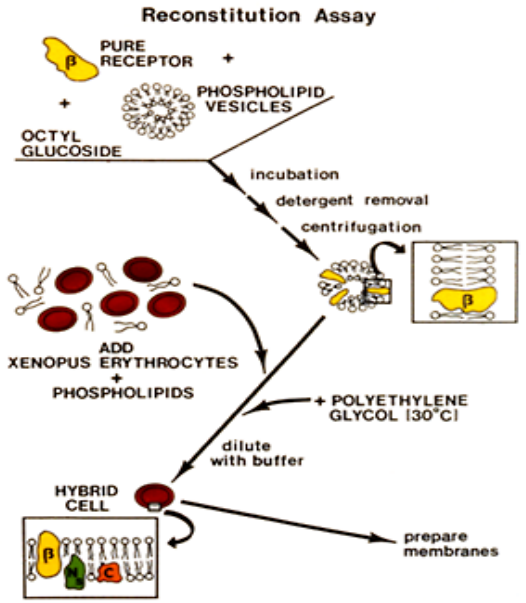
Reconstitution of a Hormone-sensitive Adenylate Cyclase System

THE PURE β-ADRENERGIC RECEPTOR AND GUANINE NUCLEOTIDE REGULATORY PROTEIN CONFER HORMONE RESPONSIVENESS ON THE RESOLVED CATALYTIC UNIT*

(Received for publication, April 18, 1984)

Richard A. Cerione,[a] David R. Sibley,[a] Juan Codina,[b,c] Jeffrey L. Benovic,[a] John Winslow,[d,e] Eva J. Neer,[d,f] Lutz Birnbaumer,[b,g] Marc G. Caron,[a] and Robert J. Lefkowitz[a,h]





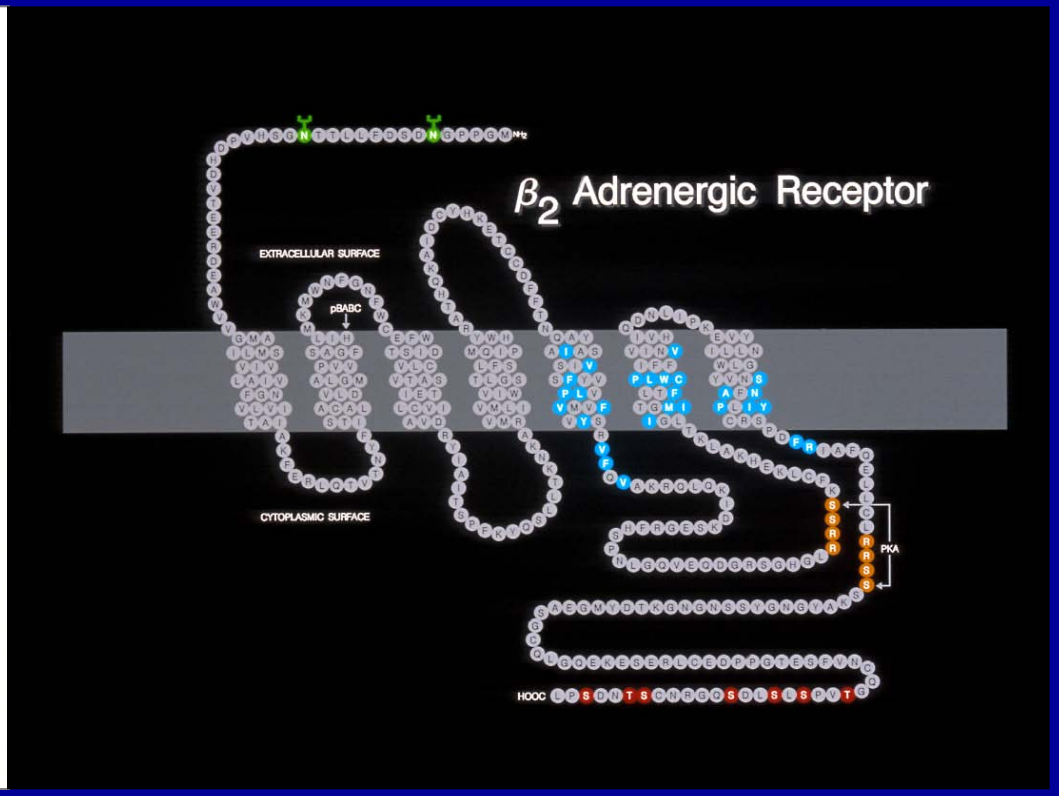
Cloning of Adrenergic Receptors

NATURE VOL. 321 1 MAY 1986 — LETTERS TO NATURE —

Cloning of the gene and cDNA for mammalian β-adrenergic receptor and homology with rhodopsin

Richard A. F. Dixon*, Brian K. Kobilka†, David J. Strader‡, Jeffrey L. Benovic†, Henrik G. Dohlman†, Thomas Frielle†, Mark A. Bolanowski†, Carl D. Bennett§, Elaine Rands*, Ronald E. Diehl*, Richard A. Mumford‡, Eve E. Slater‡, Irving S. Sigal*, Marc G. Caron†, Robert J. Lefkowitz† & Catherine D. Strader‡

Departments of *Virus and Cell Biology Research and §Medicinal Chemistry, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486, USA
† Howard Hughes Medical Institute, Department of Medicine, Biochemistry and Physiology, Duke University Medical Center, Durham, North Carolina 27710, USA
‡ Department of Biochemistry and Molecular Biology, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065, USA



Reprint Series
30 October 1987, Volume 238, pp. 650–656
SCIENCE

Cloning, Sequencing, and Expression of the Gene Coding for the Human Platelet α_2-Adrenergic Receptor

B. K. KOBILKA, H. MATSUI, T. S. KOBILKA, T. L. YANG-FENG, U. FRANCKE, M. G. CARON, R. J. LEFKOWITZ, AND J. W. REGAN

TIPS Reviews

β-Adrenergic receptors and rhodopsin: shedding new light on an old subject

Robert J. Lefkowitz, Jeffrey L.Benovic, Brian Kobilka and Marc G. Caron

Biochemistry

 Volume 26, Number 10 May 19, 1987

Perspectives in Biochemistry

A Family of Receptors Coupled to Guanine Nucleotide Regulatory Proteins

Henrik G. Dohlman, Marc G. Caron, and Robert J. Lefkowitz*

Howard Hughes Medical Institute, Departments of Medicine, Physiology, and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Received January 14, 1987; Revised Manuscript Received February 26, 1987

Regions of the Receptor Involved in Ligand & G Protein Binding

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 263, No. 31, Issue of November 5, pp. 15985–15992, 1988

Printed in U.S.A.

Site-directed Mutagenesis of the Cytoplasmic Domains of the Human β_2-Adrenergic Receptor

LOCALIZATION OF REGIONS INVOLVED IN G PROTEIN-RECEPTOR COUPLING*

(Received for publication, April 20, 1988)

Brian F. O'Dowd‡§, Mark Hnatowich‡§¶, John W. Regan‡§, W. Mark Leader‡, Marc G. Caron‡‖, and Robert J. Lefkowitz‡§

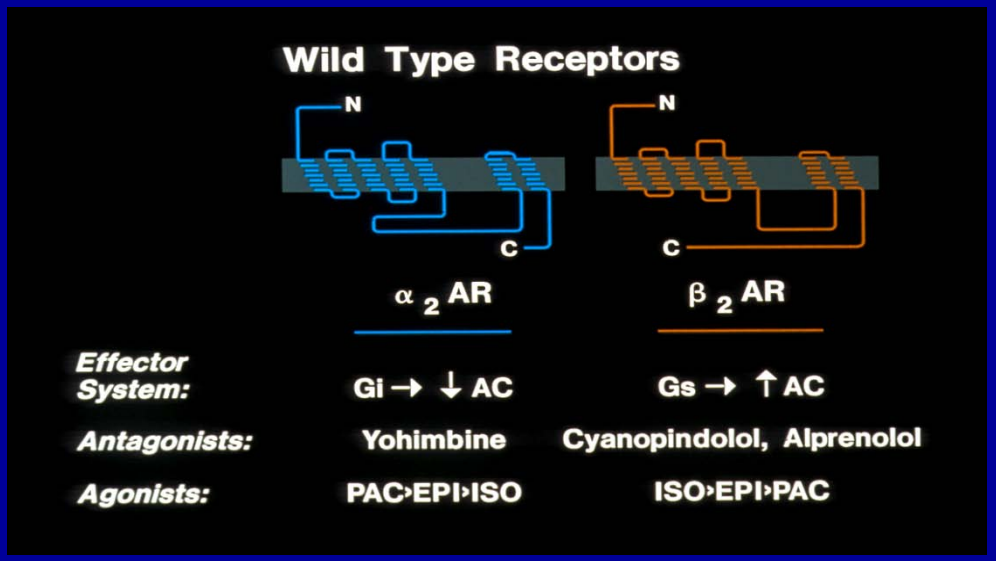
From the Departments of ‡Medicine, §Biochemistry, and ‖Cell Biology, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710

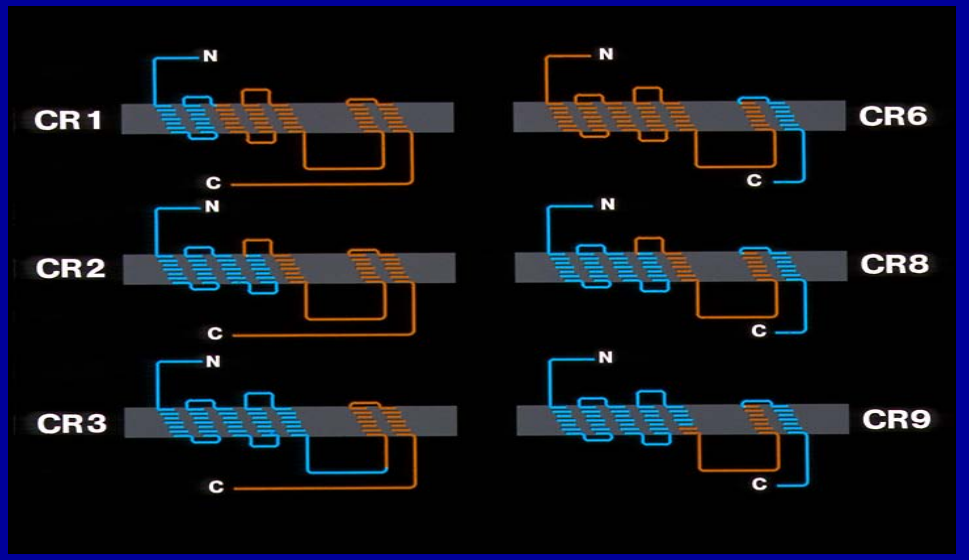
Research Articles

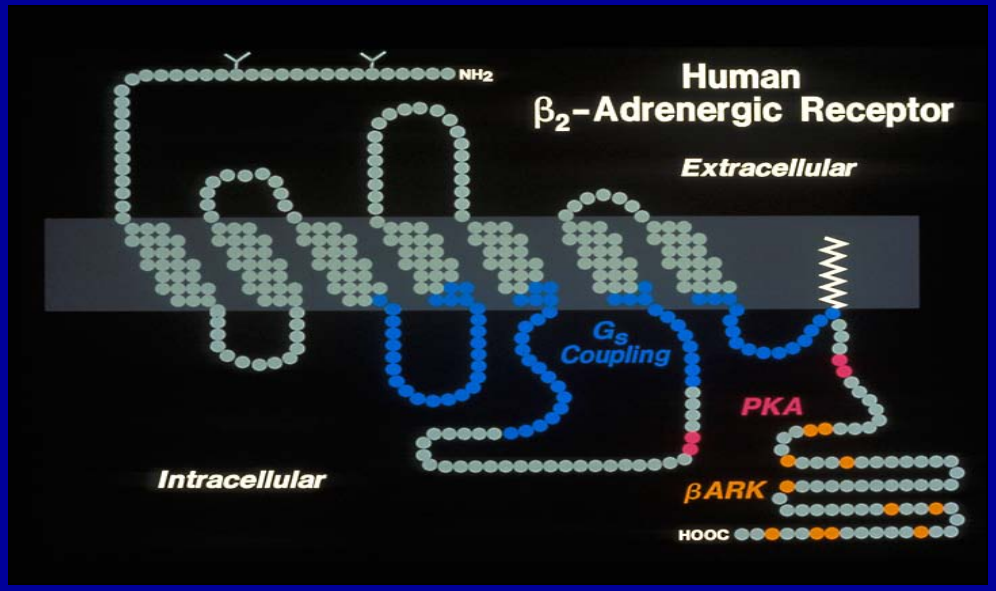
Chimeric α_2-,β_2-Adrenergic Receptors: Delineation of Domains Involved in Effector Coupling and Ligand Binding Specificity

BRIAN K. KOBILKA, TONG SUN KOBILKA, KIEFER DANIEL, JOHN W. REGAN, MARC G. CARON, ROBERT J. LEFKOWITZ

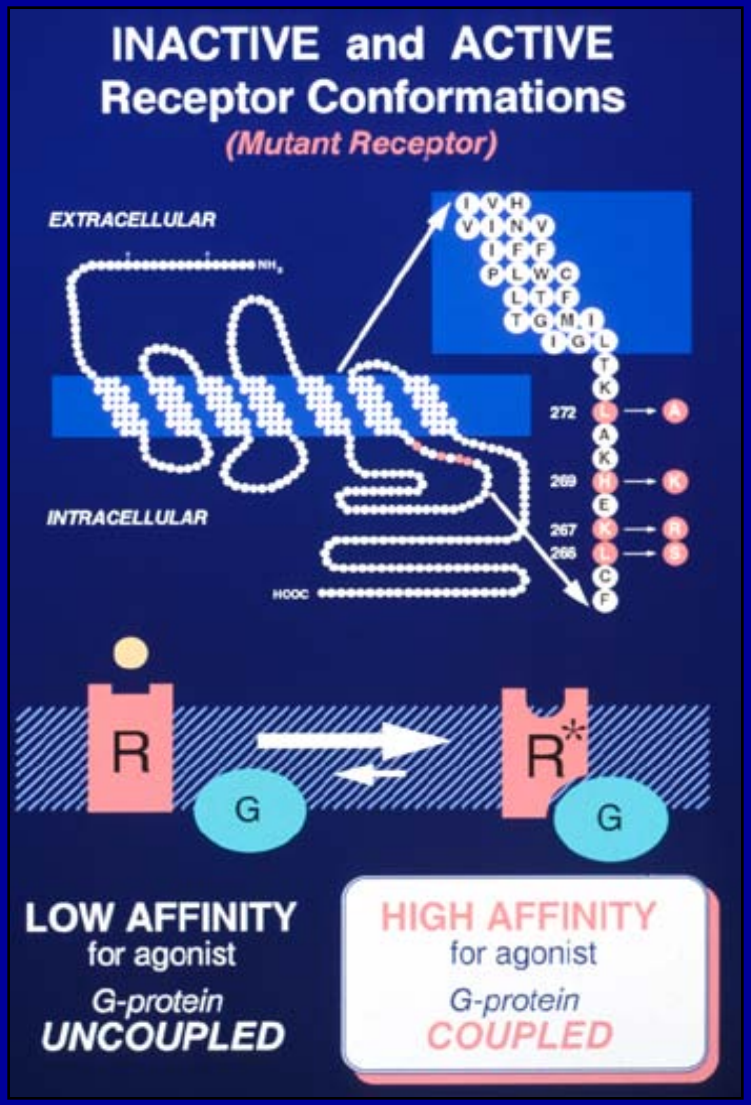
Chimeric Receptors







Constitutively Active Mutant Receptors

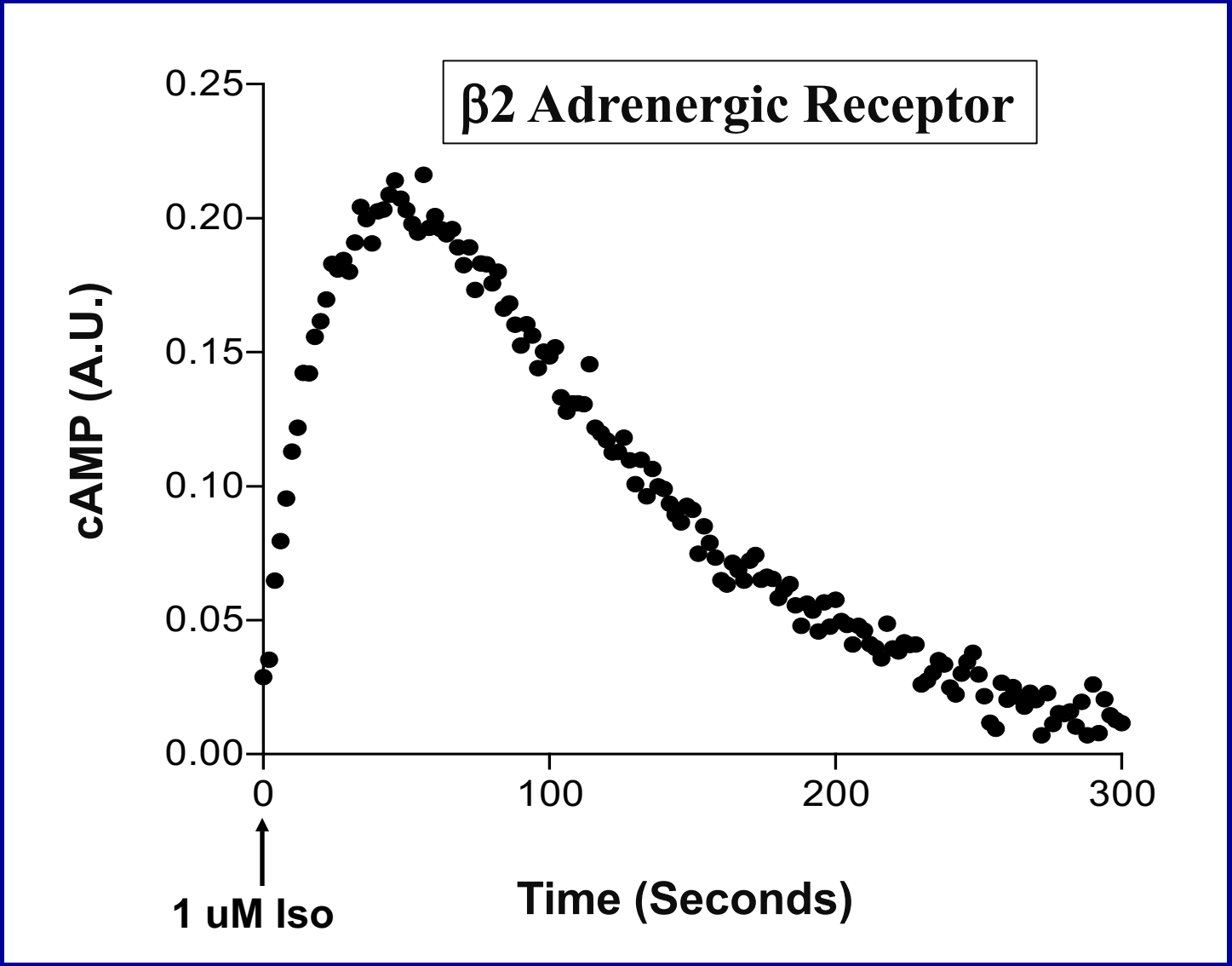


DISEASES CAUSED BY MUTATIONS OF HEPTAHELICAL RECEPTORS

Gain of Function:

Receptor	*Disease*	*Inheritance*
Rhodopsin	Cong. night blindness	Aut. dom.
LH	Familial male precocious puberty	Aut. dom.
TSH	Sporadic hyperfunctional thyroid nodules	Somatic
TSH	Familial nonautoimmune hyperthyroidism	Aut. dom.
CaR	Familial hypoparathyroidism	Aut. dom.
PTH/PTHrP	Jansen metaphyseal chondrodysplasia	Aut. dom.
FSH	Gonadotropin-independent spermatog.	Aut. dom.

Universal Mechanism of Receptor Regulation: Desensitization



Desensitization Involves Receptor Phosphorylation

Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 257, No. 16, Issue of August 25, pp. 9242–9245, 1982
Printed in U.S.A.

Catecholamine-induced Desensitization of Turkey Erythrocyte Adenylate Cyclase

STRUCTURAL ALTERATIONS IN THE β-ADRENERGIC RECEPTOR REVEALED BY PHOTOAFFINITY LABELING*

(Received for publication, February 22, 1982)

Jeffrey M. Stadel, Ponnal Nambi, Thomas N. Lavin, Sarah L. Heald, Marc G. Caron, and Robert J. Lefkowitz

From the Howard Hughes Medical Institute, Departments of Medicine (Cardiology) and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

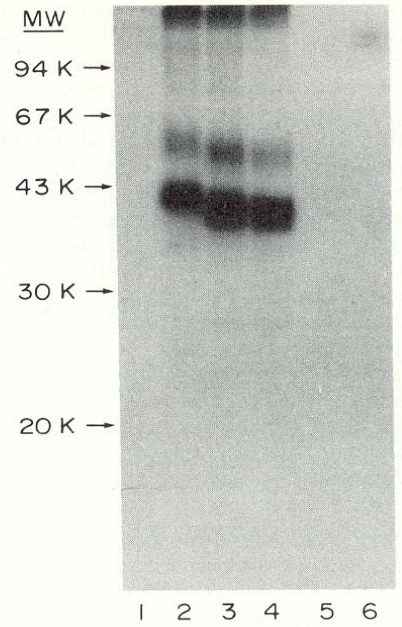


FIG. 3. **Effect of propranolol on agonist promoted-altered mobility of β-adrenergic receptor polypeptides.**

Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3173–3177, June 1983
Biochemistry

Catecholamine-induced desensitization of turkey erythrocyte adenylate cyclase is associated with phosphorylation of the β-adrenergic receptor

(protein kinase/refractoriness/8-bromoadenosine 3′,5′-cyclic monophosphate/photoaffinity labeling)

JEFFREY M. STADEL*, PONNAL NAMBI, ROBERT G. L. SHORR*, DIANE F. SAWYER, MARC G. CARON, AND ROBERT J. LEFKOWITZ†

Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 2797–2801, May 1986
Biochemistry

β-Adrenergic receptor kinase: Identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor

(S49 lymphoma cells/kin⁻ mutant/purification/desensitization/adenylate cyclase)

JEFFREY L. BENOVIC*, RUTH H. STRASSER†, MARC G. CARON‡, AND ROBERT J. LEFKOWITZ*

Howard Hughes Medical Institute, Departments of †Medicine, *Biochemistry, and ‡Physiology, Duke University Medical Center, Durham, NC 27710

β-Adrenergic Receptor Kinase: Primary Structure Delineates a Multigene Family

JEFFREY L. BENOVIC,* ANTONIO DEBLASI,† W. CARL STONE,
MARC G. CARON, ROBERT J. LEFKOWITZ

13 OCTOBER 1989 SCIENCE, VOL. 246

Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 8715–8719, October 1991
Biochemistry

The receptor kinase family: Primary structure of rhodopsin kinase reveals similarities to the β-adrenergic receptor kinase

(guanine nucleotide-binding protein-coupled receptors/desensitization/serine/threonine protein kinase/polymerase chain reaction)

WULFING LORENZ*, JAMES INGLESE*†, KRZYSZTOF PALCZEWSKI‡, JAMES J. ONORATO§¶, MARC G. CARON*‖, AND ROBERT J. LEFKOWITZ*§**

*Howard Hughes Medical Institute, Departments of §Medicine, ‖Cell Biology, †Biochemistry, Duke University Medical Center, Box 3821, Durham, NC 27710; and ‡R. S. Dow Neurological Sciences Institute of Good Samaritan Hospital and Medical Center, Portland, OR 97209

Contributed by Robert J. Lefkowitz, July 1, 1991

Cell, Vol. 74, 409–412, August 13, 1993, Copyright © 1993 by Cell Press

Minireview

G Protein-Coupled Receptor Kinases

Robert J. Lefkowitz
Departments of Medicine and Biochemistry
and the Howard Hughes Medical Institute
Duke University Medical Center
Durham, North Carolina 27710

The G Protein-Coupled Receptor Kinases (GRKs)

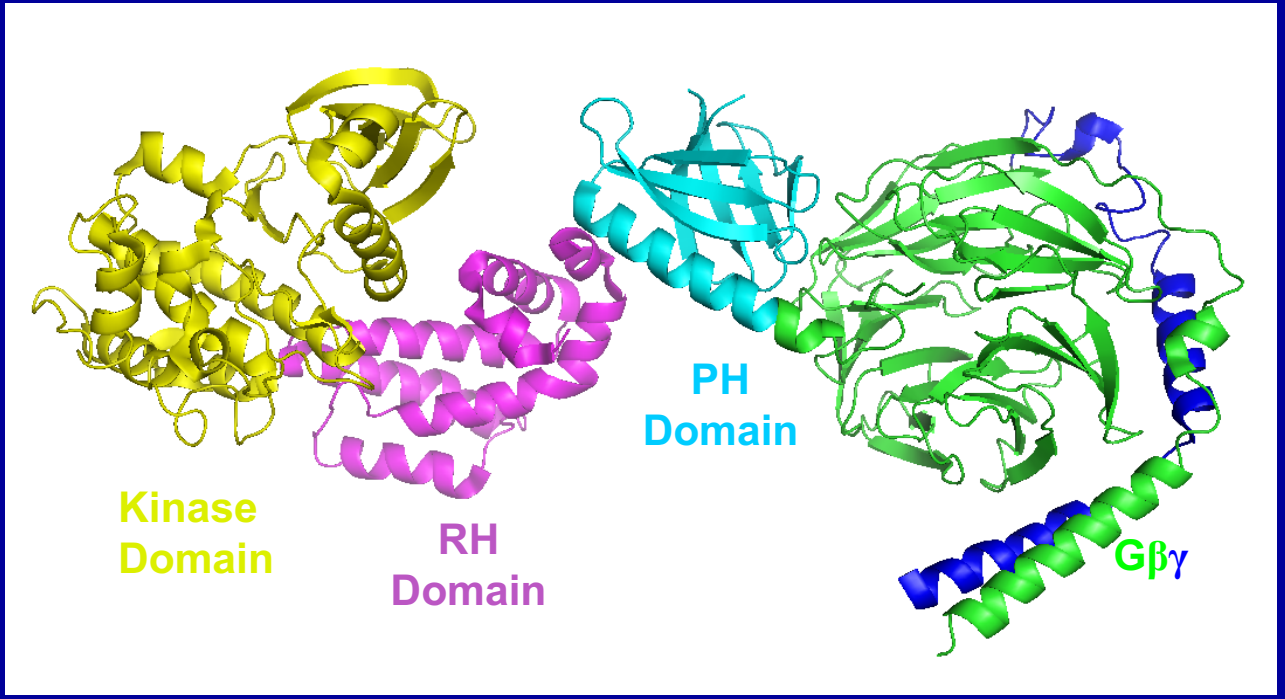
Serine/ Threonine Kinases

3 classes:

GRK1 (Rhodopsin Kinase)
GRK7

GRK2 (bARK1)
GRK3 (bARK2)

GRK4
GRK5
GRK6



Lodowski DT, Pitcher JA, Capel WD, Lefkowitz RJ, Tesmer JJ. Science, 2003, 1256-62.

Something is Missing: Discovery of β-arrestins

- Purified βARK (GRK2) loses ability to desensitize isolated β2-AR (Benovic et al '85,'86)
- Abundant retinal protein, "48 K protein" or "S Antigen" works with rhodopsin kinase to deactivate rhodopsin renamed arrestin (Kuhn, et al '87)
- "48 K protein" at high concentrations restores ability of βARK to desensitize β2-AR – (Benovic et al '87)

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 8879–8882, December 1987
Biochemistry

Functional desensitization of the isolated β-adrenergic receptor by the β-adrenergic receptor kinase: Potential role of an analog of the retinal protein arrestin (48-kDa protein)

J. L. BENOVIC*, H. KÜHN†, I. WEYAND†, J. CODINA‡, M. G. CARON*, AND R. J. LEFKOWITZ*

Discovery of β-arrestins

- **S antigen (48 kDa protein) cloned (Shinohara et al '87)**

Primary and secondary structure of bovine retinal S antigen (48-kDa protein)

(amino acid sequence/cDNA/vision/retina)

T. SHINOHARA*, B. DIETZSCHOLD†, C. M. CRAFT*, G. WISTOW*, J. J. EARLY†, L. A. DONOSO‡, J. HORWITZ§, AND R. TAO¶

- **β-arrestin1 cloned – (Lohse et al '90)**

β-Arrestin: A Protein That Regulates β-Adrenergic Receptor Function

MARTIN J. LOHSE, JEFFREY L. BENOVIC,* JUAN CODINA, MARC G. CARON, ROBERT J. LEFKOWITZ

SCIENCE, VOL. 248 REPORTS 1547 22 JUNE 1990

- **β-arrestin2 cloned – (Attramadal et al '92)**

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 267, No. 25, Issue of September 5, pp. 17882–17890, 1992
Printed in U.S.A.

β-Arrestin2, a Novel Member of the Arrestin/β-Arrestin Gene Family*

(Received for publication, April 3, 1992)

Håvard Attramadal‡, Jeffrey L. Arriza‡, Chiye Aoki§, Ted M. Dawson¶||, Juan Codina**, Madan M. Kwatra‡, Solomon H. Snyder¶, Marc G. Caron‡, and Robert J. Lefkowitz‡ ‡‡

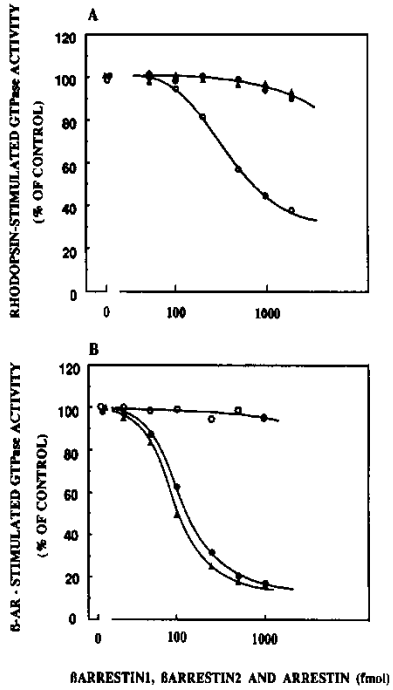
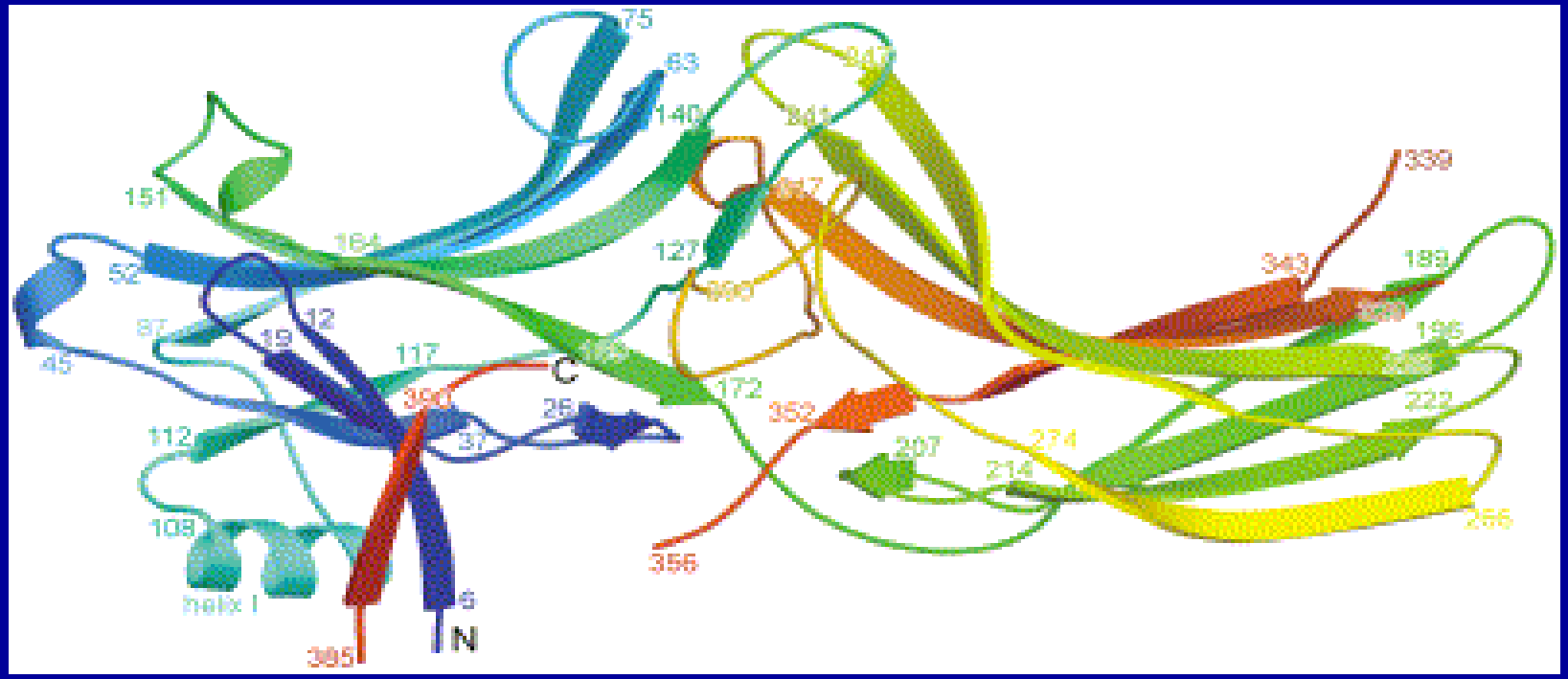


FIG. 4. Inhibition of β_2-adrenergic receptor function and rhodopsin function by β-arrestin1, β-arrestin2, and arrestin.

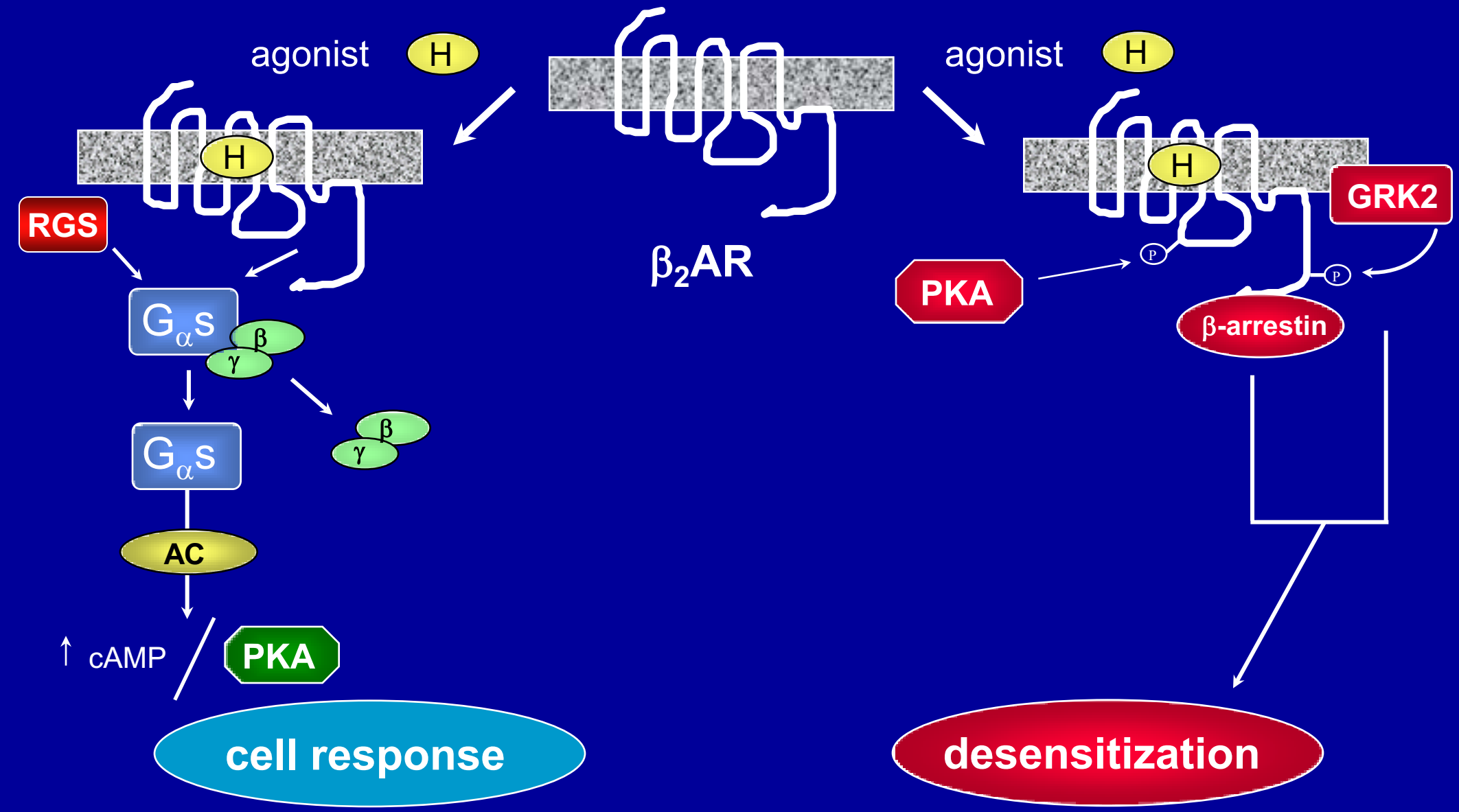
The Arrestins

	AKA	Distribution	7MSR
Arrestin 1	(Visual Arrestin)	Retinal rods	Rhodopsin
β-Arrestin 1	(Arrestin 2)	Ubiquitous	Most
β-Arrestin 2	(Arrestin 3)	Ubiquitous	Most
X Arrestin	(Arrestin 4)	Retinal cones	Opsins

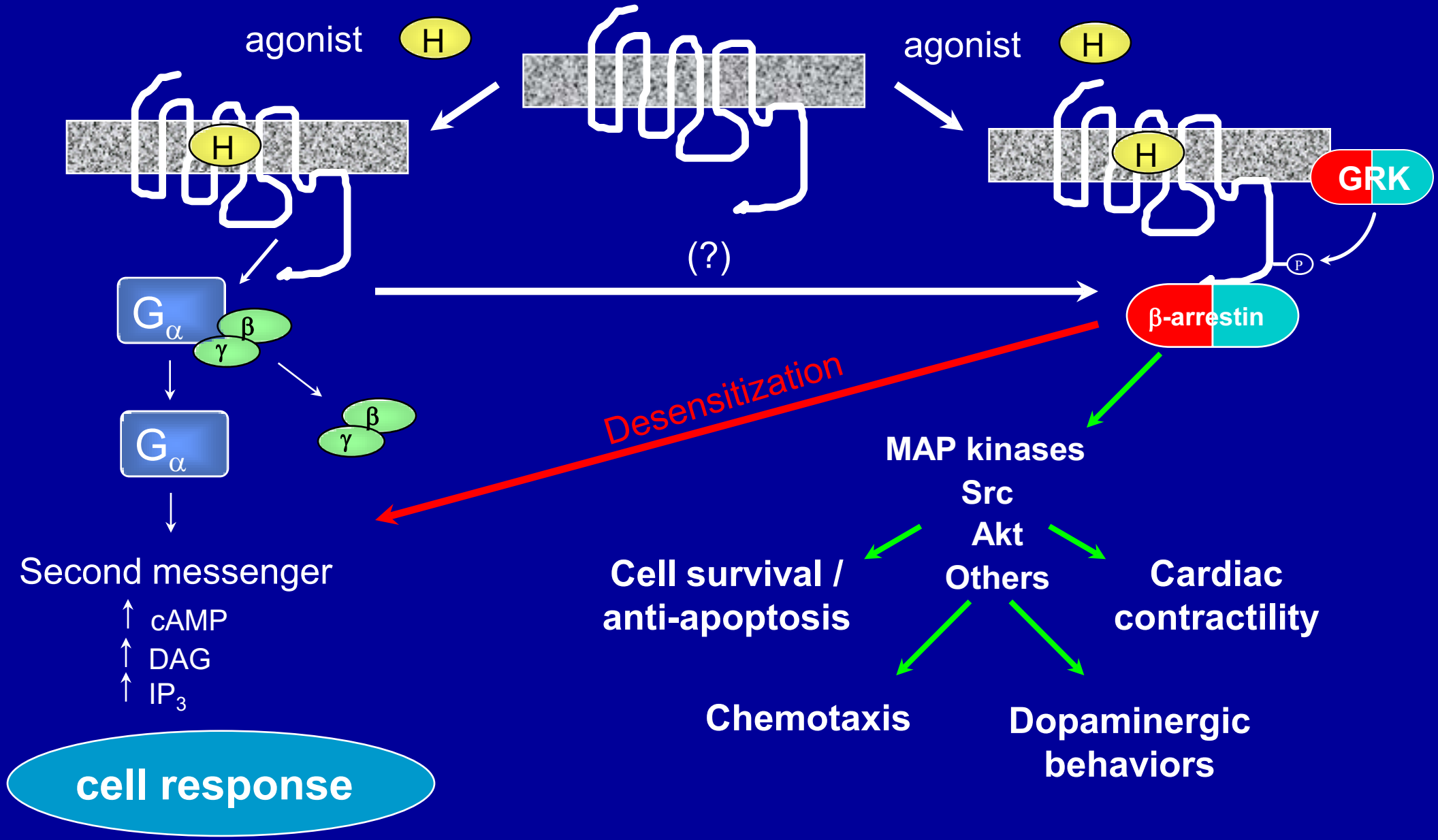


Structure solved by and figure adapted from Han M, Gurevich VV, Vishnivetskiy SA, Sigler PB & Schubert C, 2001 Structure, Vol. 9, 869–880.

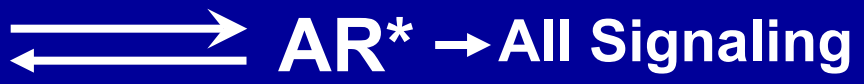
Two Paradigms: Activation & Desensitization

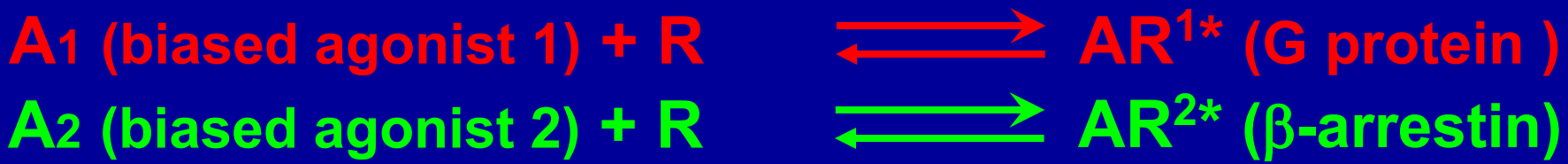


New Signaling Paradigm

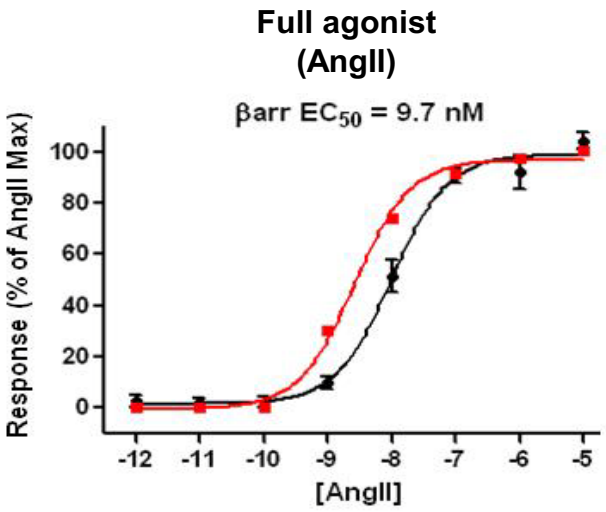


- A "**Biased Agonist**" is a ligand which stabilizes a particular active conformation of a receptor thus stimulating some responses but not others. Seven transmembrane receptor ligands, for example, can be biased toward a particular G protein or β-arrestin. Mutated receptors can also be biased.



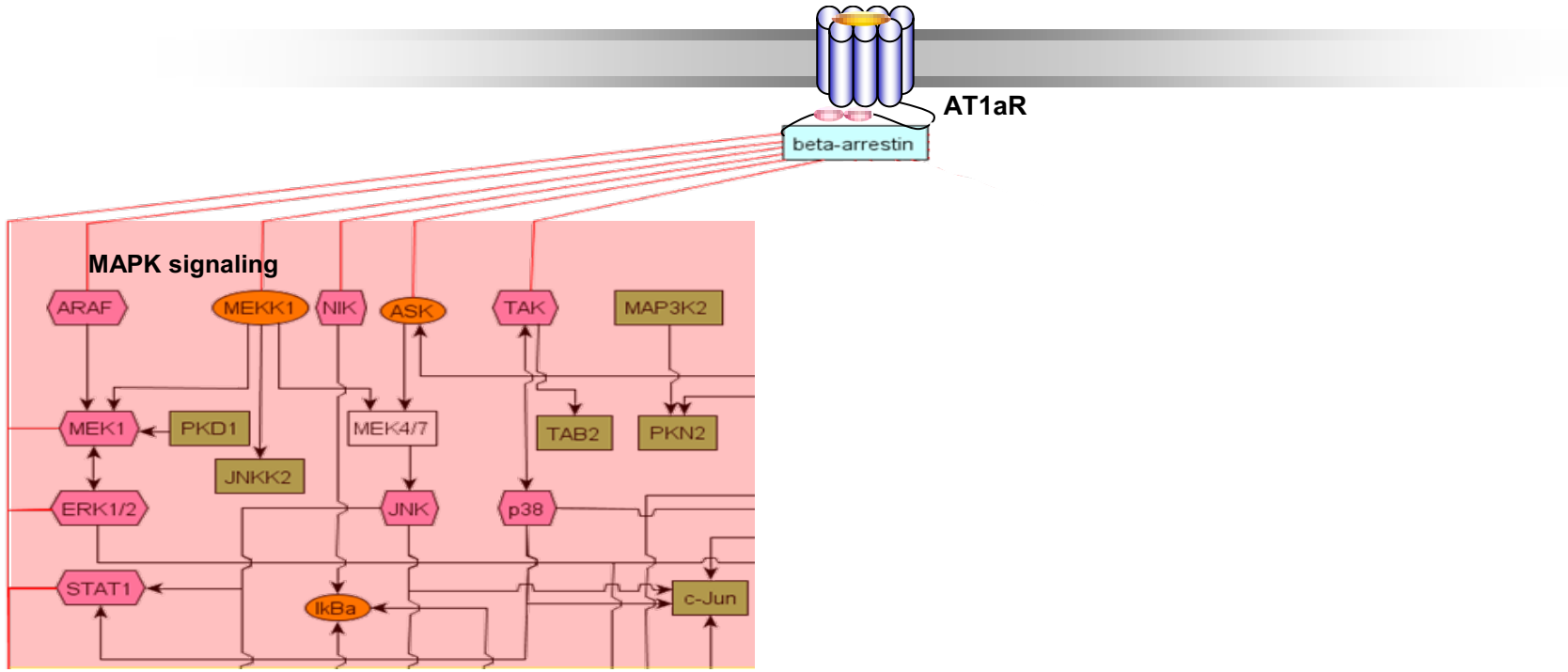


A Selective β-arrestin biased ligand at the $AT_{1A}R$



- G-protein Signal (IP1)
- B-arrestin Recruitment (PathHunter)

Quantitative, Global Phosphorylation Analysis of β-arrestin mediated Signaling



phosphoproteome

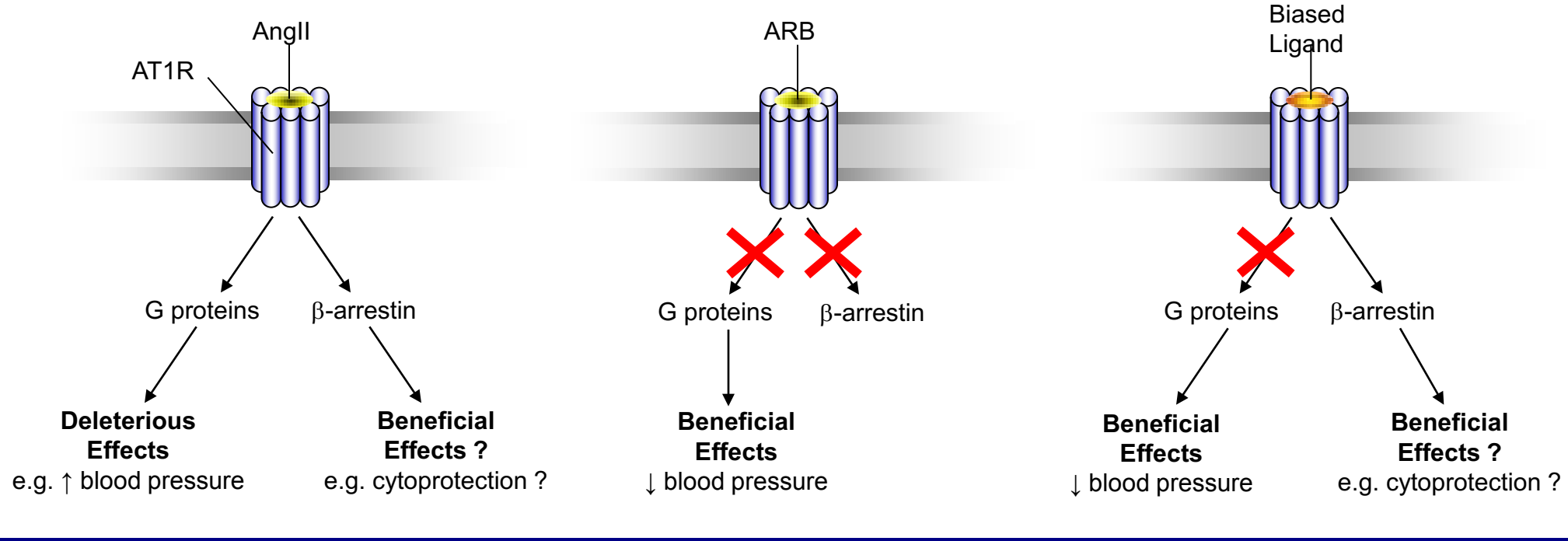
Interactome

Both

Interaction with β-arrestin

Phosphorylation Regulation

A "biased ligand" at the $AT_{1A}R$ signals only through β-arrestin



Violin & Lefkowitz, TiPS 2007

Ligands which are biased toward either β-arrestin or G-Protein Signaling have Potential Therapeutic Benefit

7TMR	Example	Direction of Bias	Advantage
Opioid Receptor	TRV120027	G-Protein	• Reduced side effects such as constipation, respiratory depression • Lowers blood pressure • Decreased tolerance • Increases cardiac performance
			• Antiapoptotic

Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence

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β-arrestin2 mediates anti-apoptotic signaling through regulation of bad phosphorylation

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Selective activation of β-arrestin-1 ligands at the AT1R reduces blood pressure and increases cardiac performance

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