ECS Overview

& Endocannabinoidome

Patients Out of Time

15th National Clinical Conference on Cannabis Therapeutics

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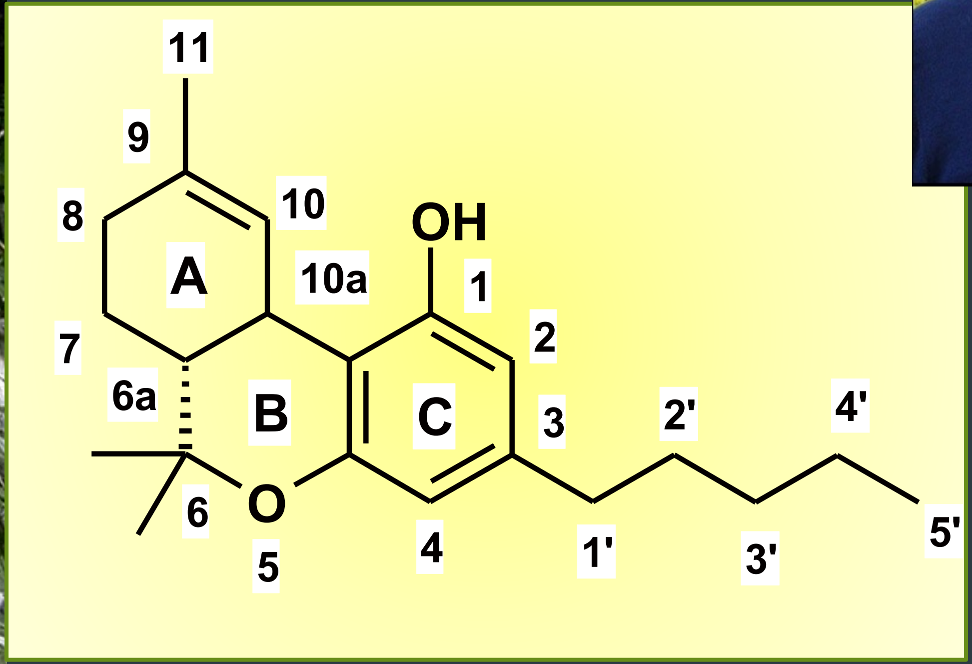
*Dr. Howlett has no conflicts of interest to declare.

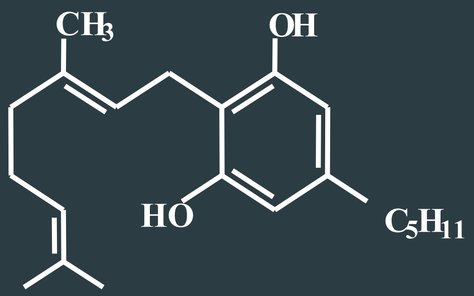
1. Introduction: The Endocannabinoid System & Beyond
2. The Endocannabinoid System Components
   a. CB_1 and CB_2 cannabinoid receptors
      Agonists, Antagonists, Allosteric modulators
   b. Endocannabinoids anandamide and 2-arachidoylglycerol
   c. Enzymes of endocannabinoid synthesis
   d. Enzymes of endocannabinoid biotransformation
3. Cellular Signaling to achieve Active Responses
   a. Cellular Signaling in the ECS
      Example describing the regulation of neurotransmission
   b. Associated Proteins
      G-proteins; β-arrestins
      CRIP1a; SGIP; BiP; GASP
4. Summary and Conclusions

Identification of Marijuana's CNS-active Compound Δ^9-Tetrahydrocannabinol (THC)

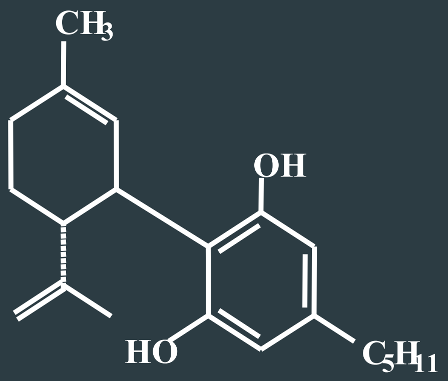


Mechoulam R, Hanuš LO, Pertwee R, Howlett AC. Early phytocannabinoid chemistry to endocannabinoids and beyond. Nat Rev Neurosci. 2014

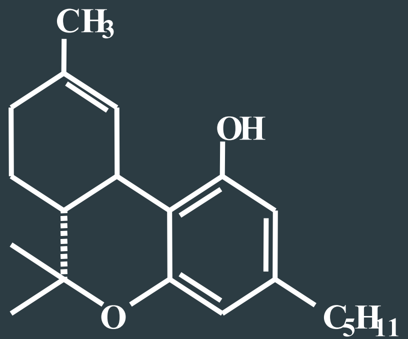
Other phytocannabinoids



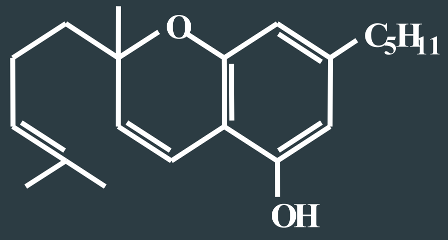
cannabigerol (CBG)
(Gaoni and Mechoulam, 1964)



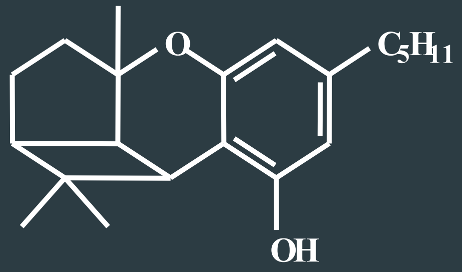
cannabidiol (CBD)
(Mechoulam and Shvo, 1963)



Δ^9-tetrahydrocannabinol (Δ^9-THC)
(Gaoni and Mechoulam, 1964)



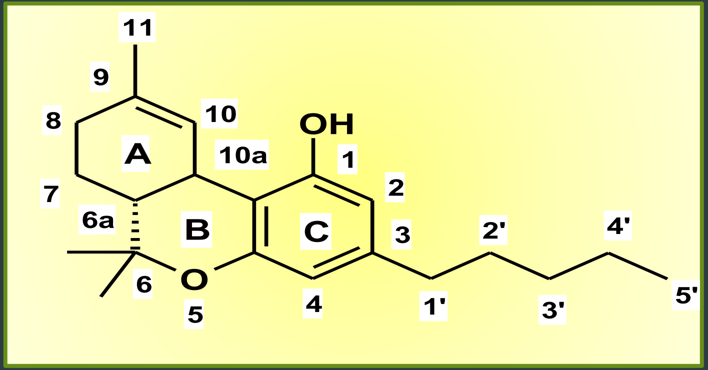
cannabichromene (CBC)
(Claussen et al., 1966;
Mechoulam and Gaoni, 1966)

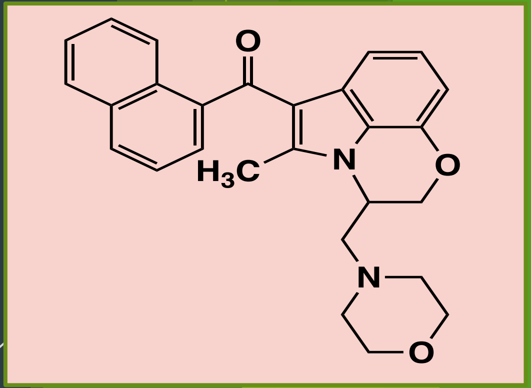


cannabicyclol (CBL)
(Crombie et al., 1968)

First Generation pharmaceutical design was based on Δ9-THC analogs

- Tetrahydrocannabinol (THC)
- Pharmaceutical Industry interest in developing cannabinoid drugs, currently used extensively in preclinical research studies
  - Cannabinoid: levonantradol
  - Nonclassical cannabinoid: CP55940





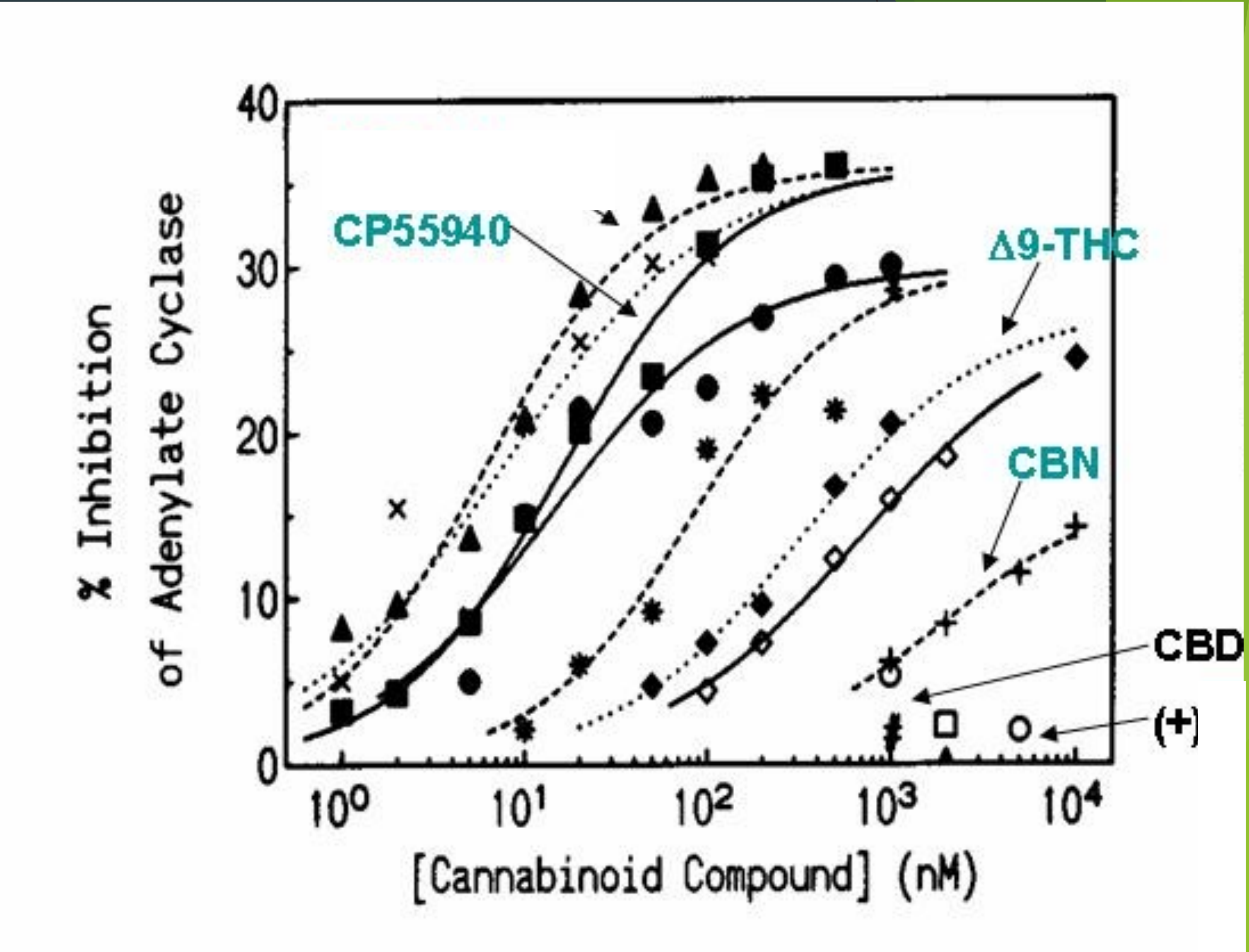
Amino alkyl indole: WIN55212-2

Mechoulam R, Hanuš LO, Pertwee R, Howlett AC. (2014) Nat Rev Neurosci. 15, 757.
Howlett AC, Thomas BF, Huffman JW (2021) Molecules 26, 6190.

Agonists inhibit cAMP production in neuronal cells

- CP55940 is a full agonist.
- Δ9-THC is a partial agonist compared with CP55940.
- CBN is a weak partial agonist.
- CBD is not active.

Mukhopadhy et al. (2002) Chem Phys Lipids 121:91



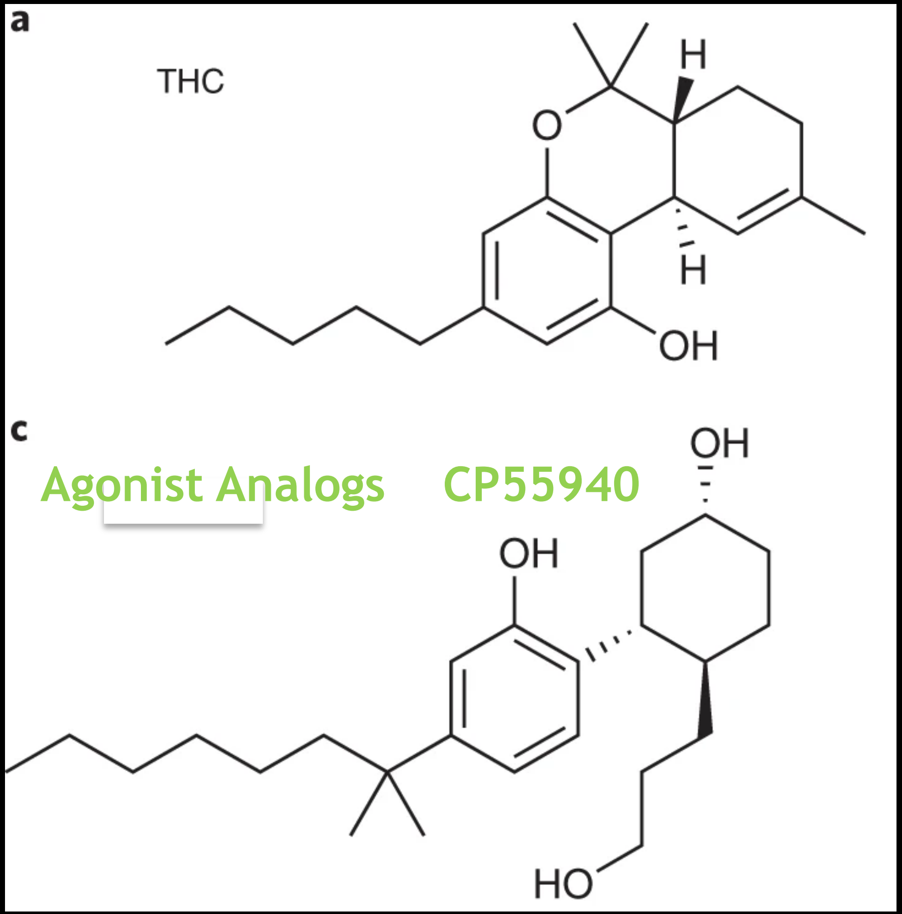
The Target for Δ^9-THC is the Endocannabinoid System

- Research identified a cellular mechanism for Δ^9-THC as the activation of CB_1 and CB_2 cannabinoid receptors.
- Endogenous lipid mediators, known as "endocannabinoid" ligands, stimulate CB_1 and CB_2 cannabinoid receptors in normal physiology.
- Synthetic and biotransformation enzymes regulate the cellular production, release and removal of endocannabinoid ligands.

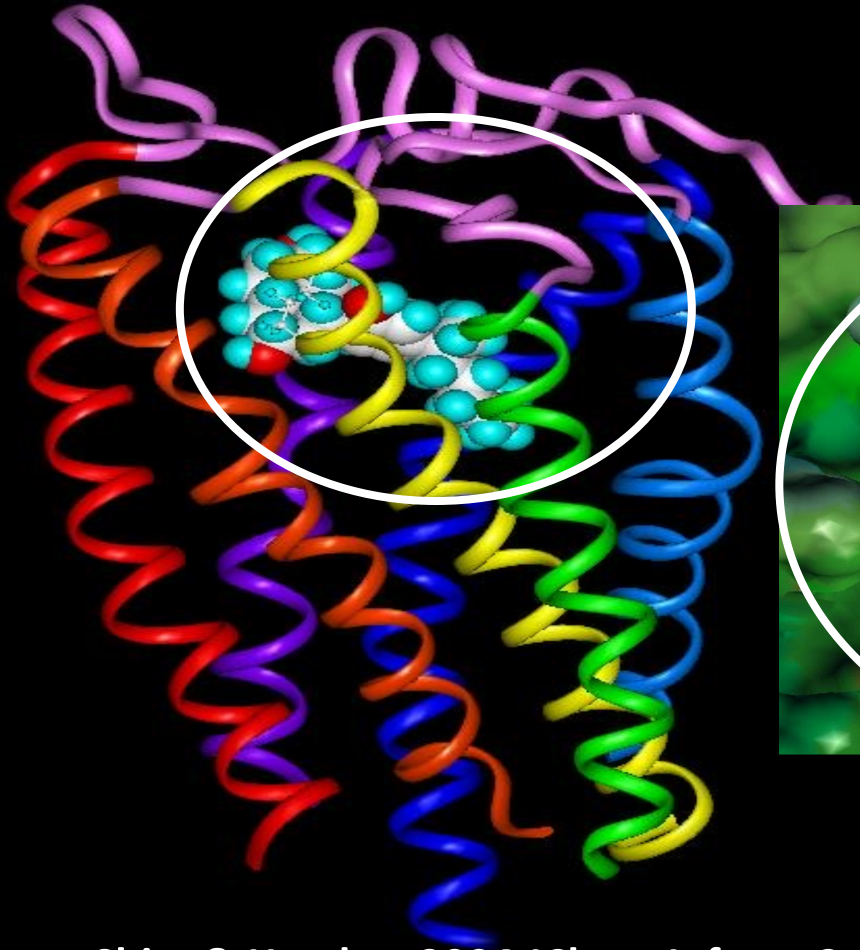
Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R and Pertwee RG (2002) **Classification of Cannabinoid Receptors**, Pharmacol. Rev. 54:161.
Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, and Ross RA (2010) **Cannabinoid Receptors and their Ligands: Beyond CB_1 and CB_2.** Pharmacol. Rev. 62:588-631.

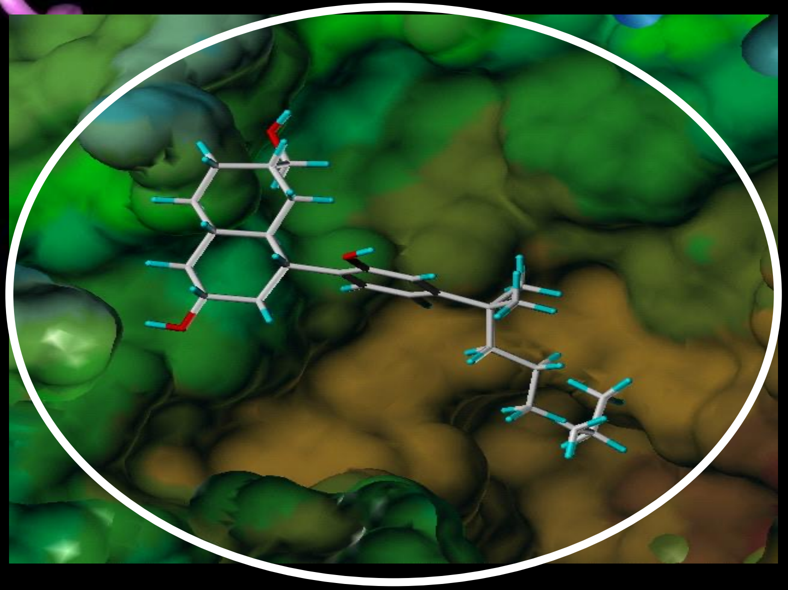
Abood M, Alexander SP, Barth F, Bonner TI, Bradshaw H, Cabral G, Casellas P, Cravatt BF, Devane WA, Di Marzo V, Elphick MR, Felder CC, Greasley P, Herkenham M, Howlett AC, Kunos G, Mackie K, Mechoulam R, Pertwee RG, Ross RA. **Cannabinoid receptors (version 2019.4) in the IUPHAR/BPS Guide to Pharmacology Database**. IUPHAR/BPS Guide to Pharmacology CITE. 2019; 2019(4). Available from: https://doi.org/10.2218/gtopdb/F13/2019.4.

CB_1 and CB_2 Cannabinoid Receptors are 7-transmembrane proteins that bind cannabinoid agonists and endocannabinoids



extracellular



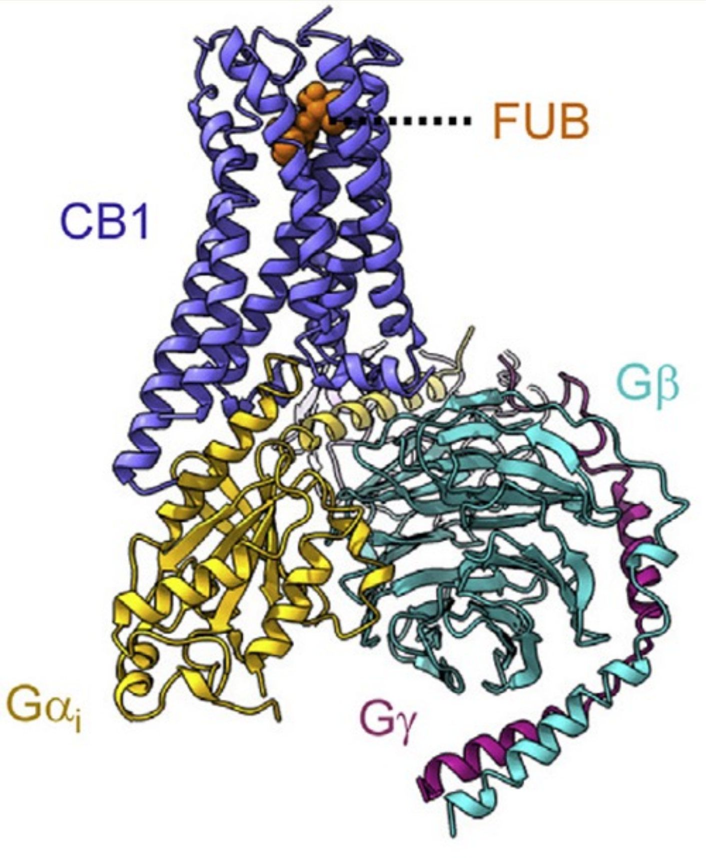


intracellular

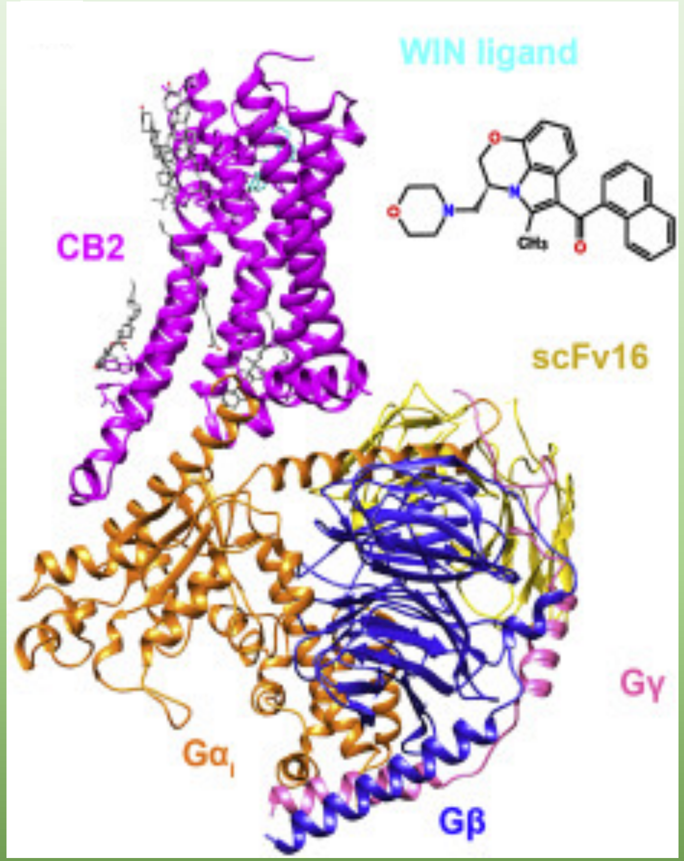
Shim & Howlett 2004 JChem Inform Comput Sci 44:1466

Shim, Welsh, and Howlett 2003 Biopolymers71:169

CB1 and CB2 Receptors couple to Gαi1-β1γ2 to activate a response

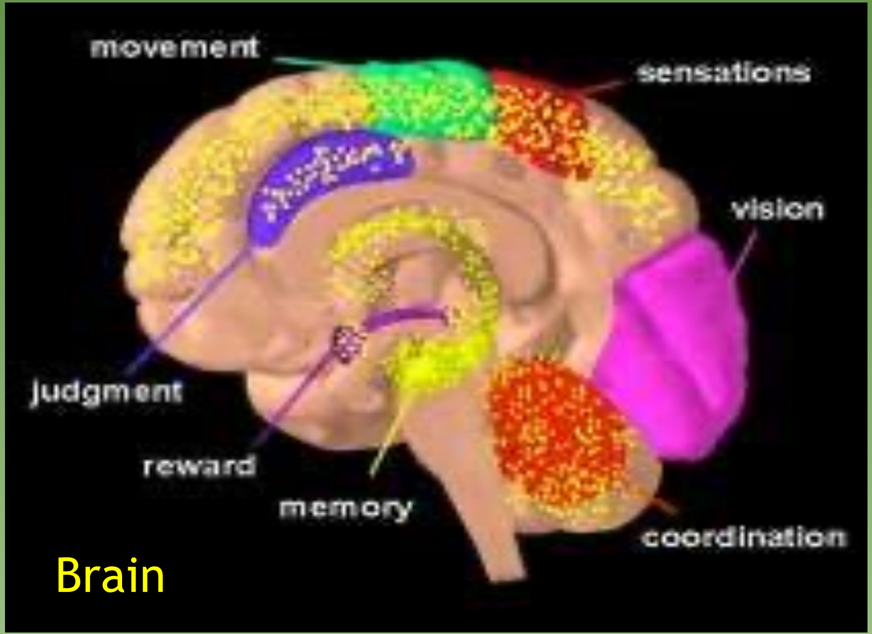


Krisha Kumar et al., 2019 Cell 176:448

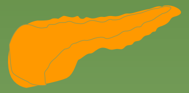


Xing et al., 2020 Cell 180:645

CB1 Cannabinoid Receptors in Brain and other Organs

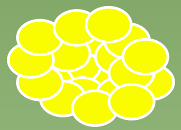


Liver
Hepatocytes



Endocrine
Pancreas

Skeletal
Muscle



Adipose
Tissue

GI
Tract

Bone

CB1 selective agonists: **Arachidonylcyclopropylamide**
Arachidonyl-(2)-chloroethylamide
O-1812

Cristino, Palomba, Di Marzo 2014Int J Obes 4(Suppl 1):S26; Gatta-Cherifi, Cota 2015 Handb Exp Pharmacol. 231:367; Goodman, Packard 2015 Neurobiol Learn Mem. 125:1; Gruden, Barutta, Kunos, Pacher 2016 Br J Pharmacol. 173:1116; Lee et al. 2016 Am J Physiol Gastrointest Liver Physiol. 311:G655; Mallat, Teixeira-Clerc, Lotersztajn 2013 J Hepatol. 59:891; Manzanares et al. 2018 Biochem Pharmacol.157:108; Mastinu et al. 2018 Horm Mol Biol Clin Investig. 36(2); O'Sullivan2015 Handb Exp Pharmacol.231:393; Pertwee 2012 Philos TransR Soc Lond B Biol Sci.367:3353; Rossi et al. 2019 Int J Mol Sci. 20:1919; Rubino, Zamberletti, Parolaro 2015 Handb Exp Pharmacol.231:261; Sharkey and Wiley 2016 Gastroenterology. 151:252; Silveira et al. 2017 Neurosci Biobehav Rev.76(Pt B):380

CB2 Cannabinoid Receptors in the Immune System

Spleen, Tonsils

B and T Cells

Macrophage and Monocytic cells

- **Brain Microglia**
- **Bone Osteoclasts**
- **Liver Kupffer cells**

Brain: Neuroprogenitor cells, oligodentrocyte progenitor cells

Neurons in some loci in the brain (brainstem, cerebellum)

CB2 selective agonists: JWH-133; AM1241; HU-308

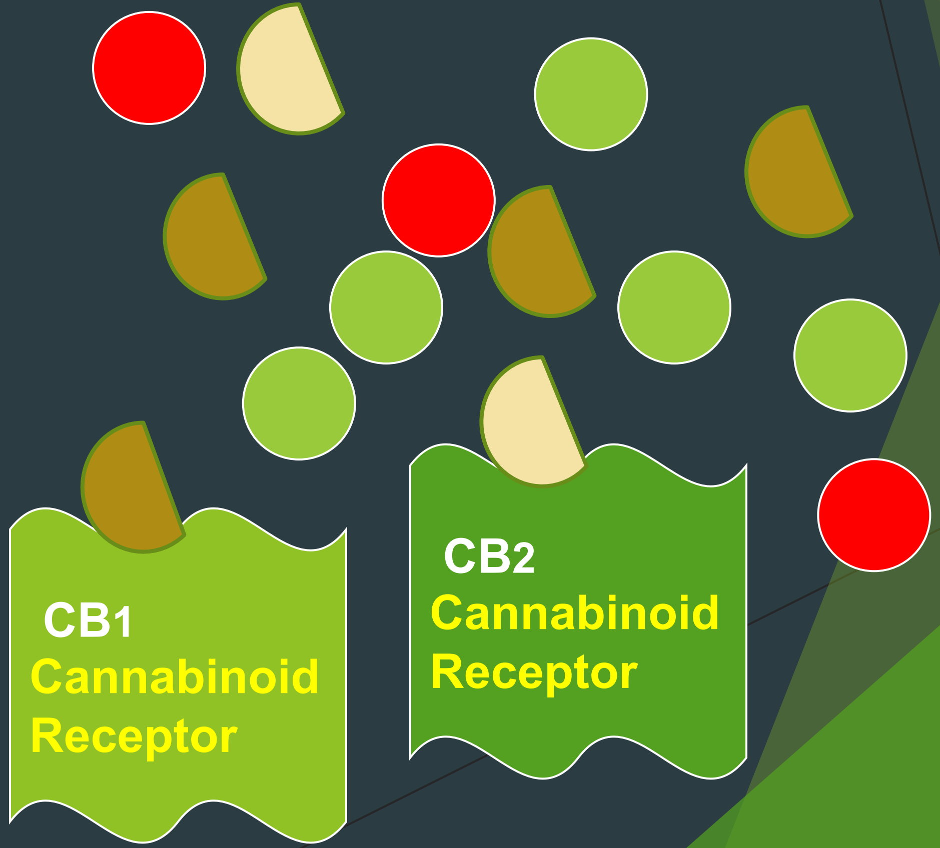
Cabral and Staab 2005 In: Pertwee R.G. (eds) Cannabinoids. Handbook Exper Pharmacol 168.
Stella 2010 Glia 58

Competitive Antagonists: Compete for binding to Cannabinoid Receptors but fail to cause a response.

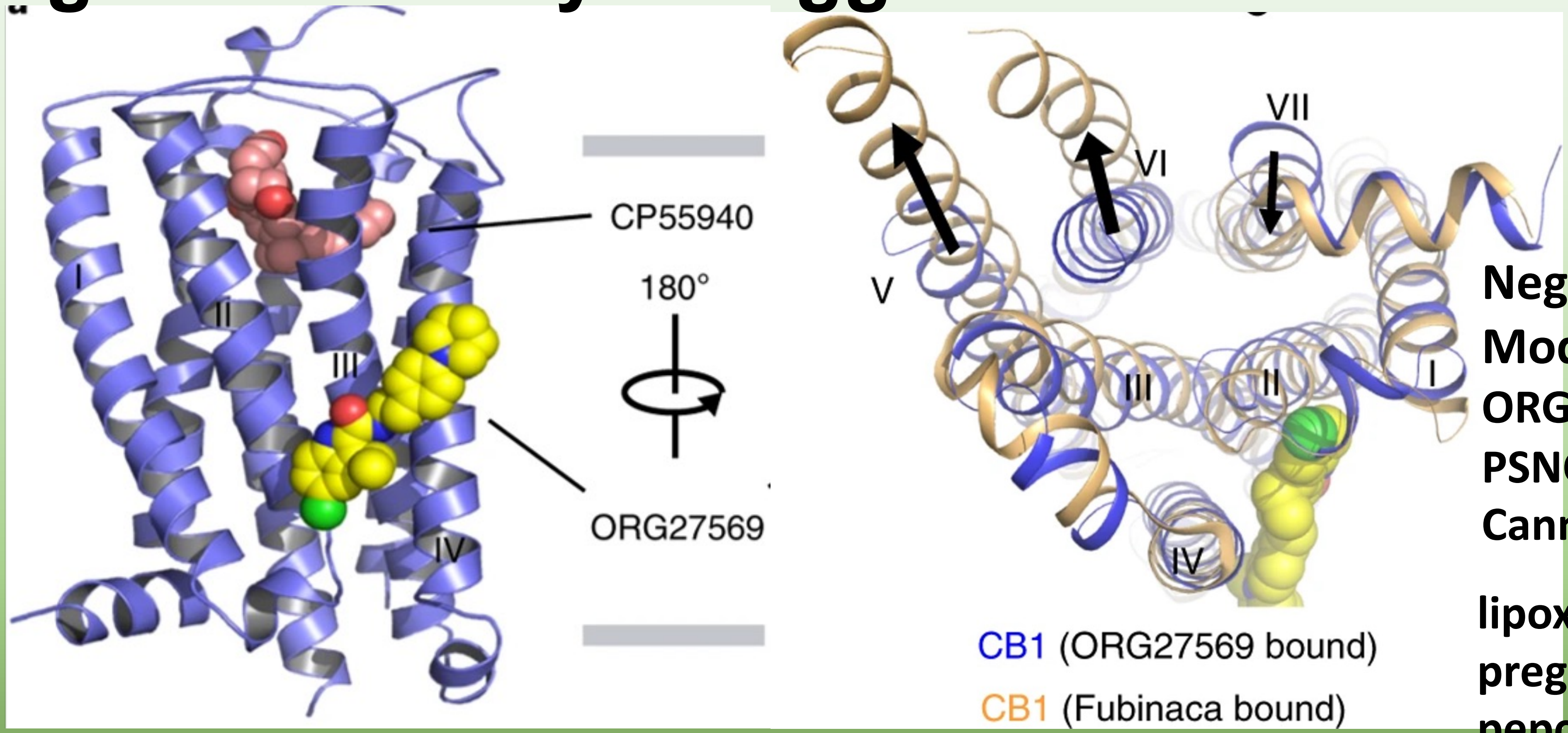
SR141716 (rimonabant) **competitive selective CB1 antagonist**

SR144528 **competitive selective CB2 antagonist**

- CB1 competitive antagonists
  - SR141716 (rimonabant)
  - AM251, AM281, AM6545
  - LY320135
- CB2 competitive antagonists
  - SR144528
  - AM630



Negative Allosteric Modulators (NAMs) block agonist's ability to toggle into active mode



Negative Allosteric Modulators (NAMs): ORG27569, PSNCBAM1, Cannabidiol (CBD)

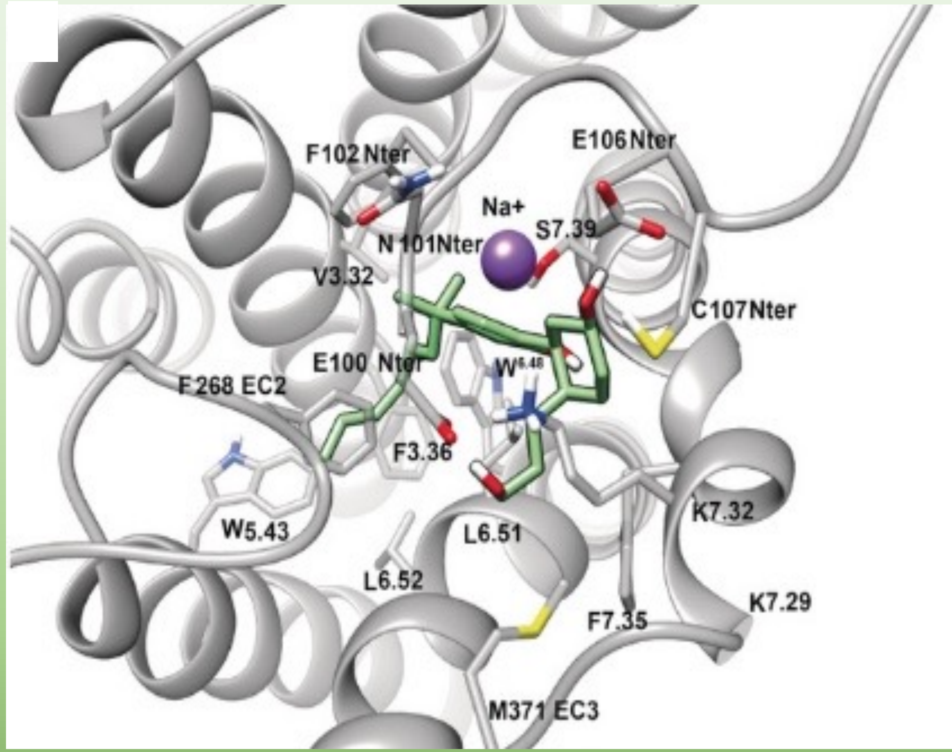
lipoxinA4, pregnenolone, pepcans

Shao et al., 2019 Nat. Chem. Biol. 15:1199

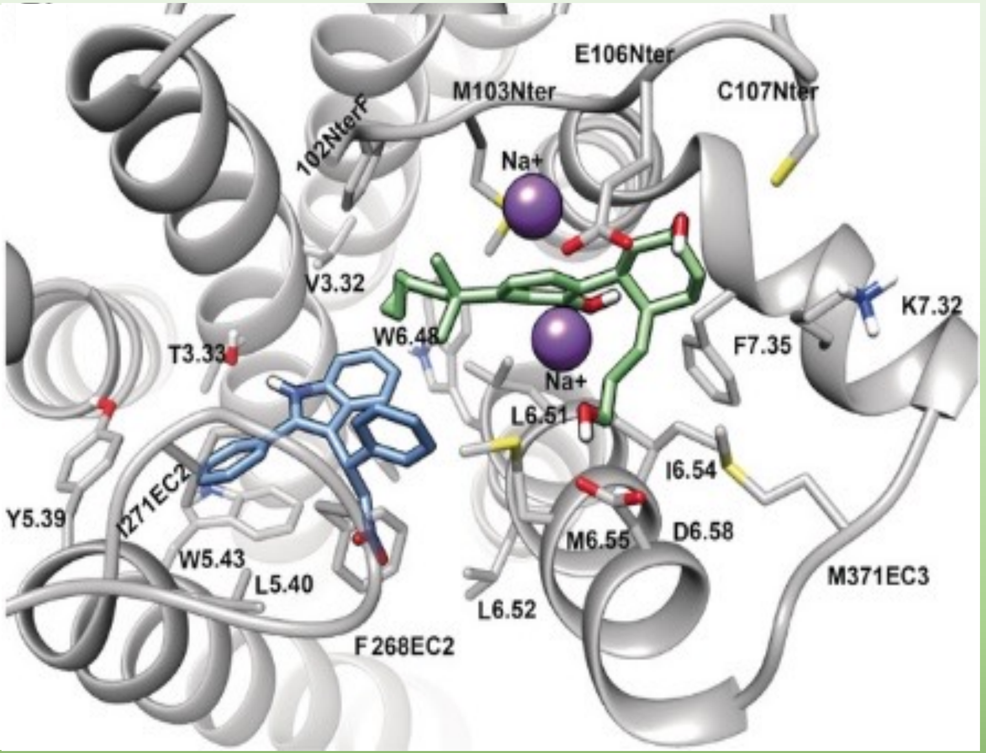
Khurana, Mackie, Piomelli and Kendall 2017 Neuropharmacol.124:3

Positive Allosteric Modulators (PAMs) facilitate agonist binding and activation energy

CP55940 alone



GAT228 opens channel for **CP55940**



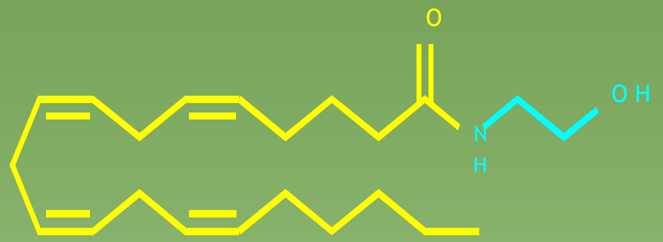
Positive Allosteric Modulators (PAMs)
GAT211, GAT228, GAT229,ZCZ011

Saleh et al. 2018 Angew. Chem. Int. Ed. 57: 2580

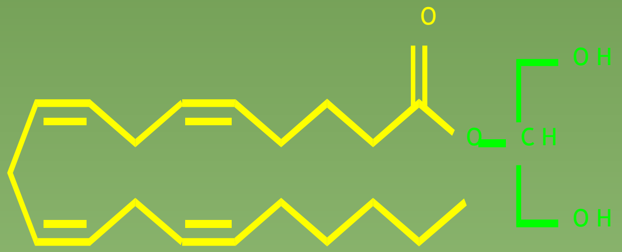
Khurana, Mackie, Piomelli and Kendall 2017Neuropharmacol.124:3

Endocannabinoids stimulate both CB1 and CB2 cannabinoid receptors

Arachidonylethanolamide (Anandamide)



2-Arachidonoylglycerol (2-AG)

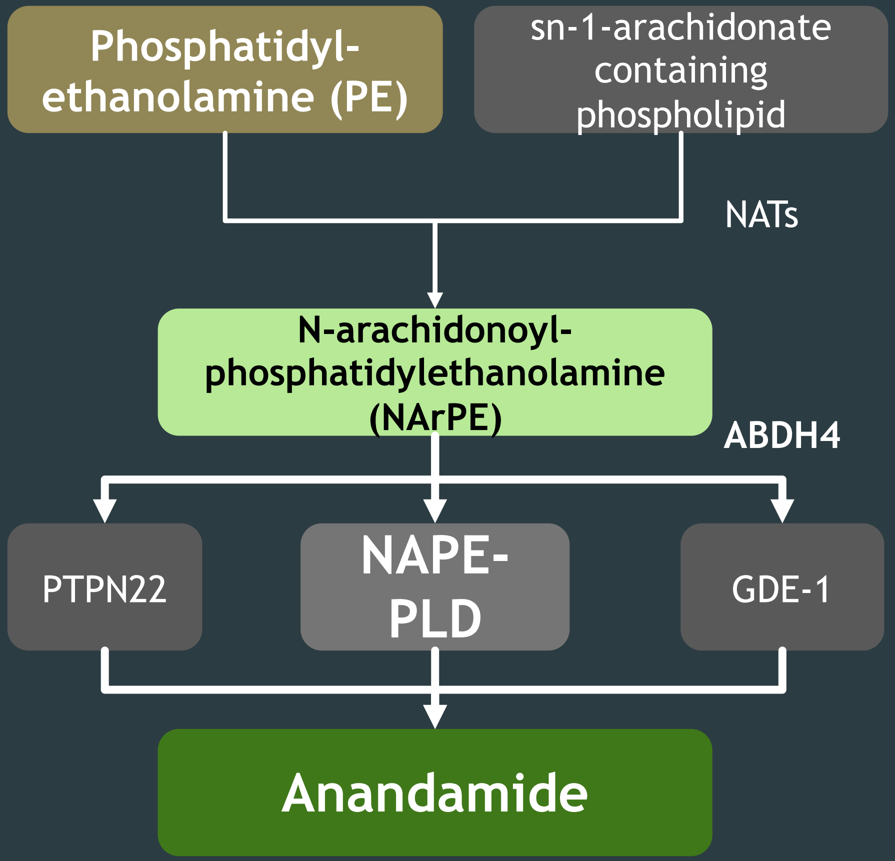


Partial Agonist-Antagonist
Alone is an agonist, but would inhibit a full-agonist

Full Agonist

Blankman et al. 2013 Pharmacol Rev. 65:849.

How Endocannabinoids are synthesized



- NAPE-PLD Inhibitors
- Decrease Anandamide
  - hexachlorophene, bithionol
  - LEI-401
  - ARN-19874

Iannotti et al. 2016 Prog Lipid Res. 62:107

How Endocannabinoids are synthesized

Phosphatidic acid (PA)

Phospholipid

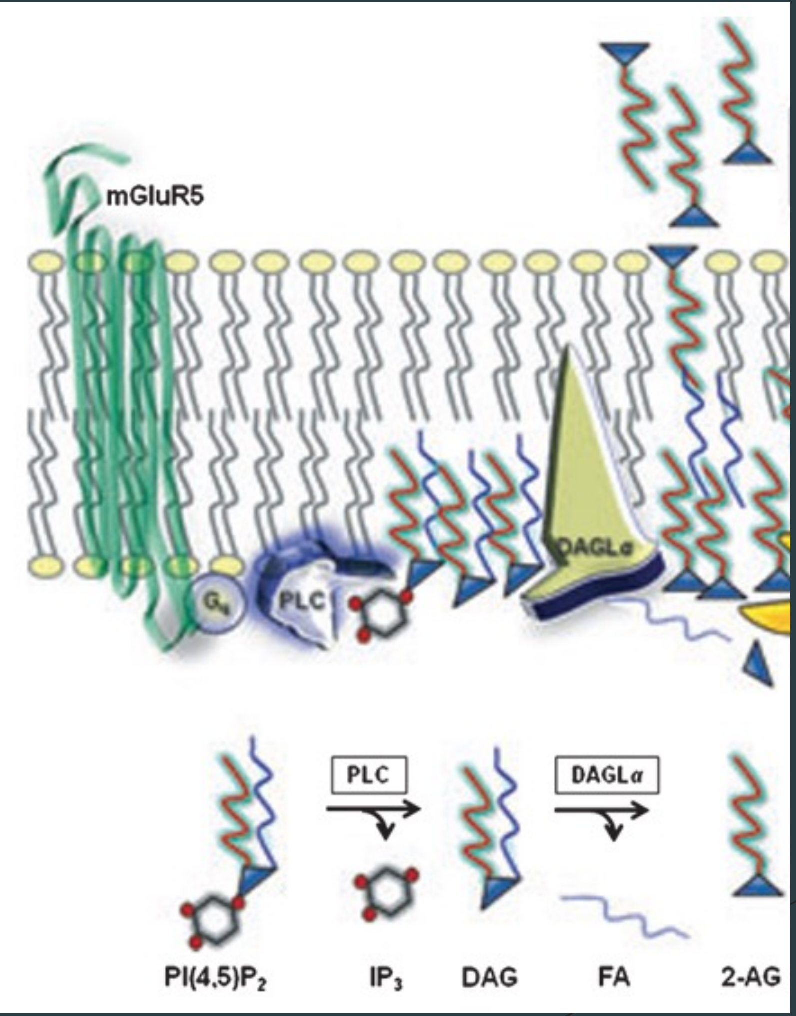
PA phosphohydrolase

PLCβ

sn-2-arachidonate-diacylglycerol

DAGL a/β

2-AG



- Diacylglycerol Lipase Inhibitors
- Decrease 2-AG

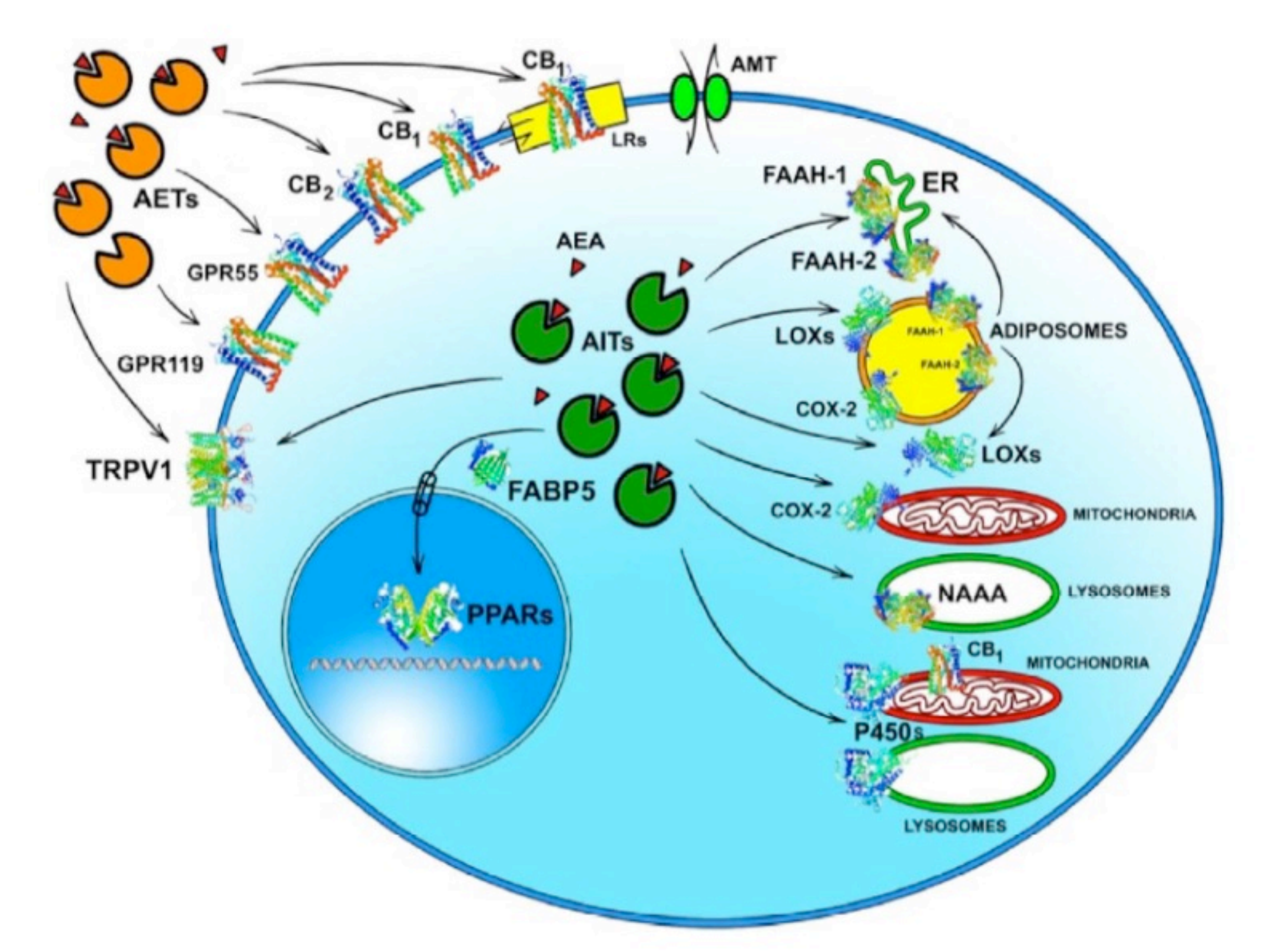
tetrahydrolipstatin (Orlistat)

RHC80267

LEI-105

Iannotti et al. 2016 Prog Lipid Res. 62:107

Promiscuity of Anandamide and Acyl-Ethanolamides



Maccarrone (2017) Front. Mol. Neurosci. 10:166

Arachidonyl ethanolamide

Oleyl ethanolamide

Palmityl ethanolamide

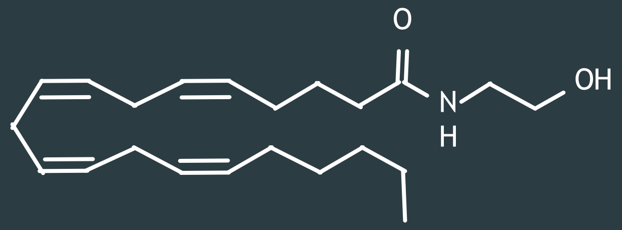
Cyclooxygenase 2 (COX-2) uses endocannabinoids as substrates to create prostamides and further derivatives that are bioactive lipids.

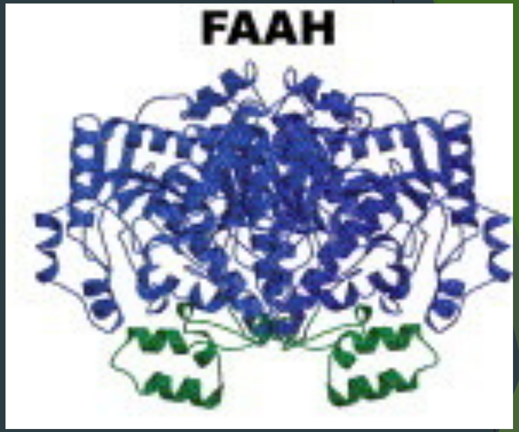
Lipoxygenase and P450 Oxidative enzymes use endocannabionds as substracts.

Metabolism of Anandamide and other AEAs

Fatty Acid Amide Hydrolase

Anandamide

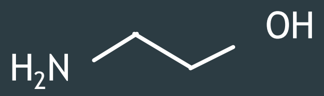




ER enzymes { FAAH-1
FAAH-2

Lysosomal enzyme – NAAA

→ Ethanolamine



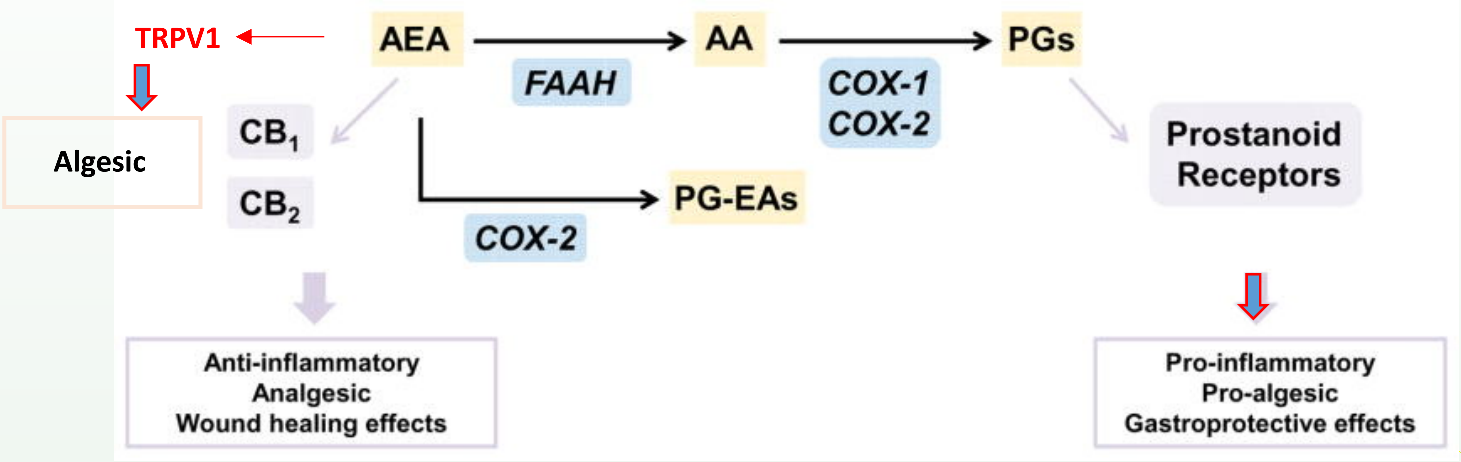
Arachidonic acid (AA)

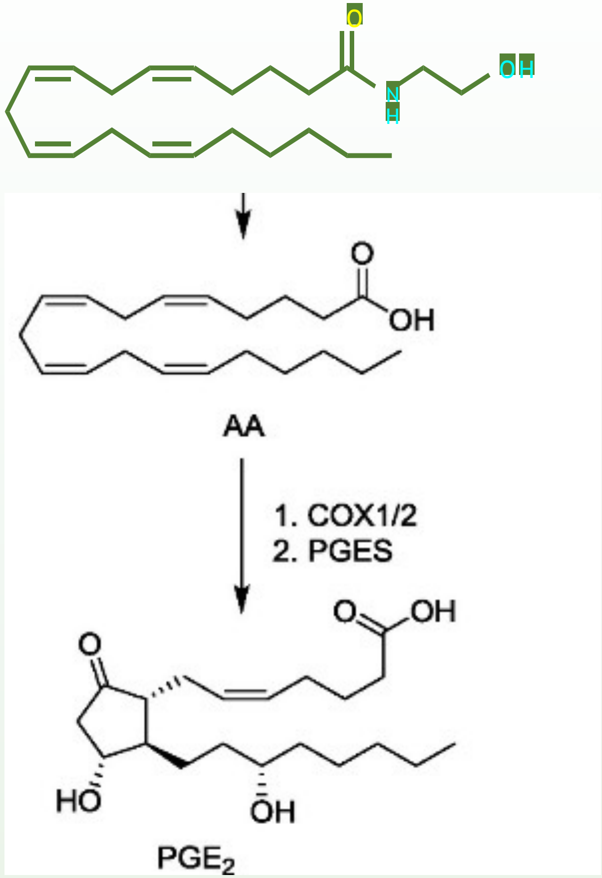
Cravatt et al. 2004 FEBS Letters 567: 159

Blankman,Cravatt 2013 Pharmacol Rev 65:849

Maccarrone M. *Front Mol Neurosci.* 2017;10:166.

Metabolism of Anandamide





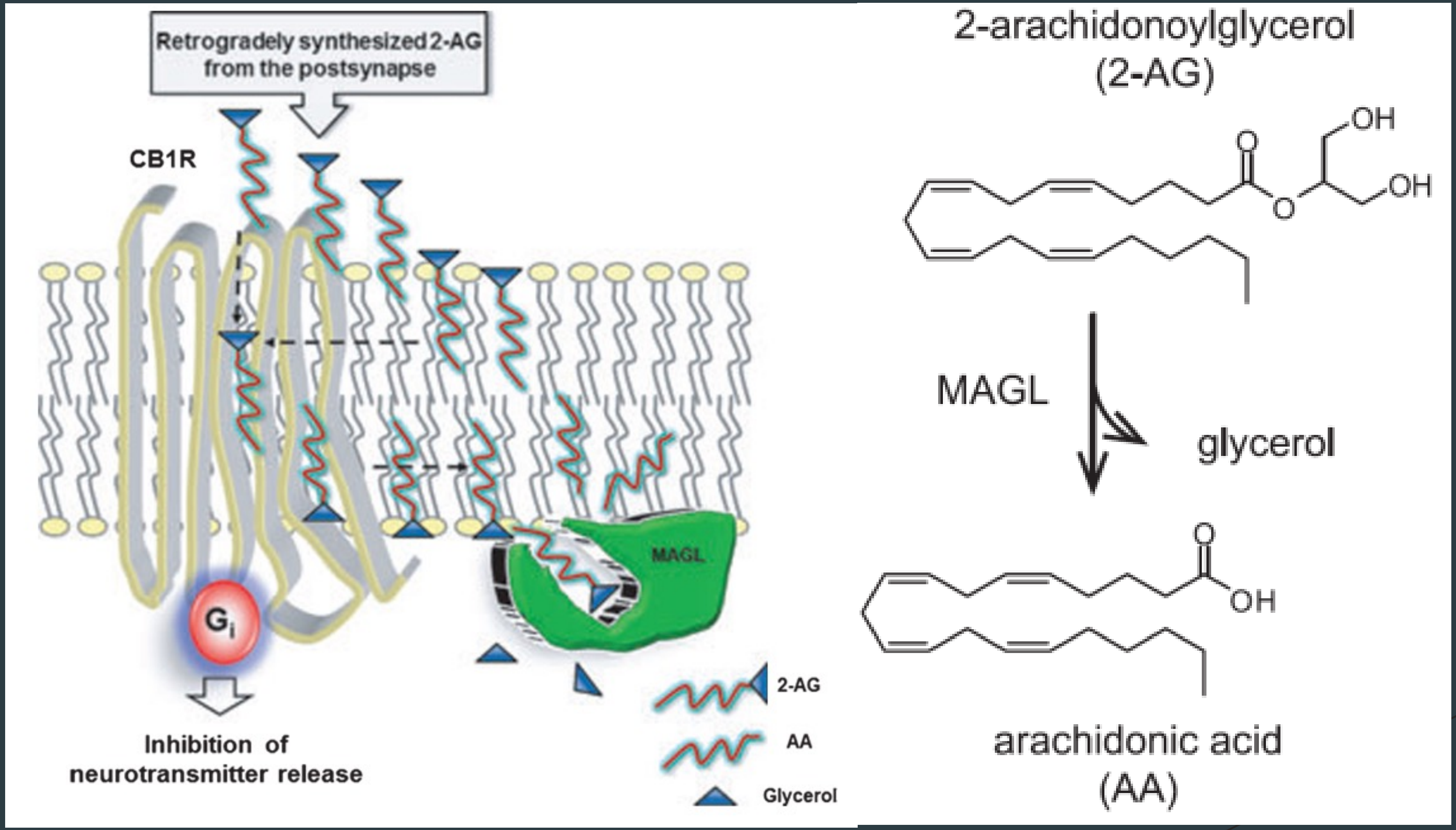
Cyclooxygenase 2 (COX-2) Endocannabinoids act as substrates to create prostamides and further derivatives that are bioactive lipids.

Inhibition of 2-AG or Anandamide catabolism to arachidonic acid reduces substrate availability for COX2 in inflammatory sites where COX2 is induced.

Fowler et al., Br J Pharmacol. 2009
Scarpelli et al., ChemMedChem. 2016

Metabolism of 2-AG *Mono-AcylGlycerol Lipase*

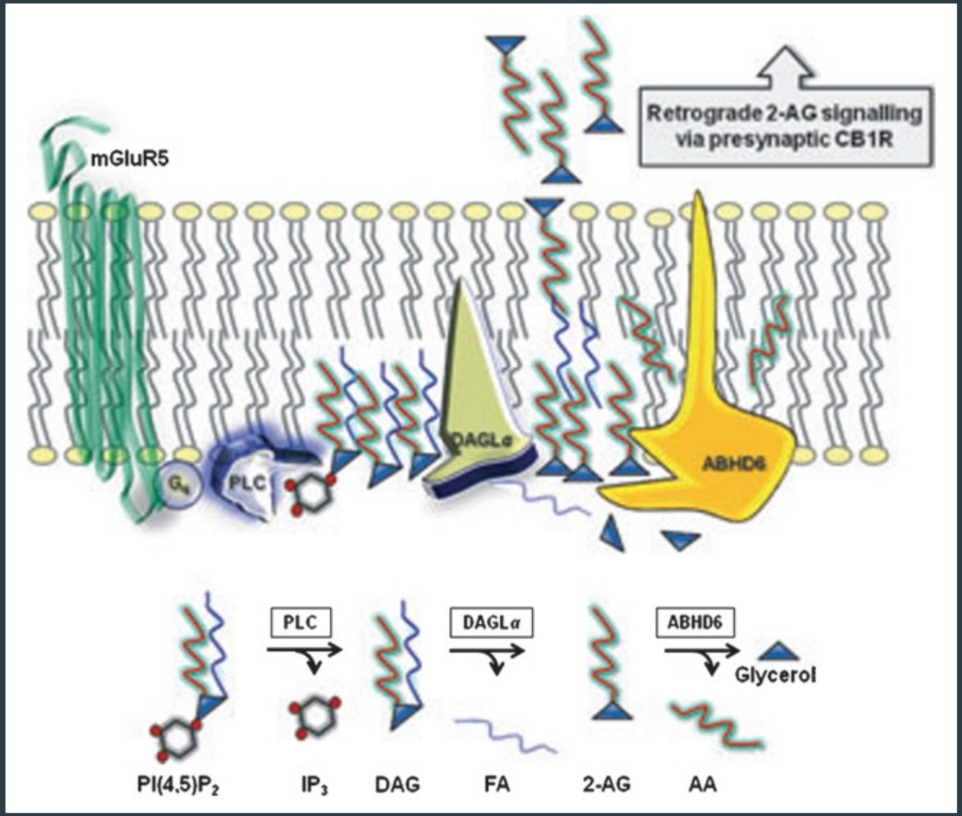
- MAGL Clears Presynaptic 2-AG As It Accumulates

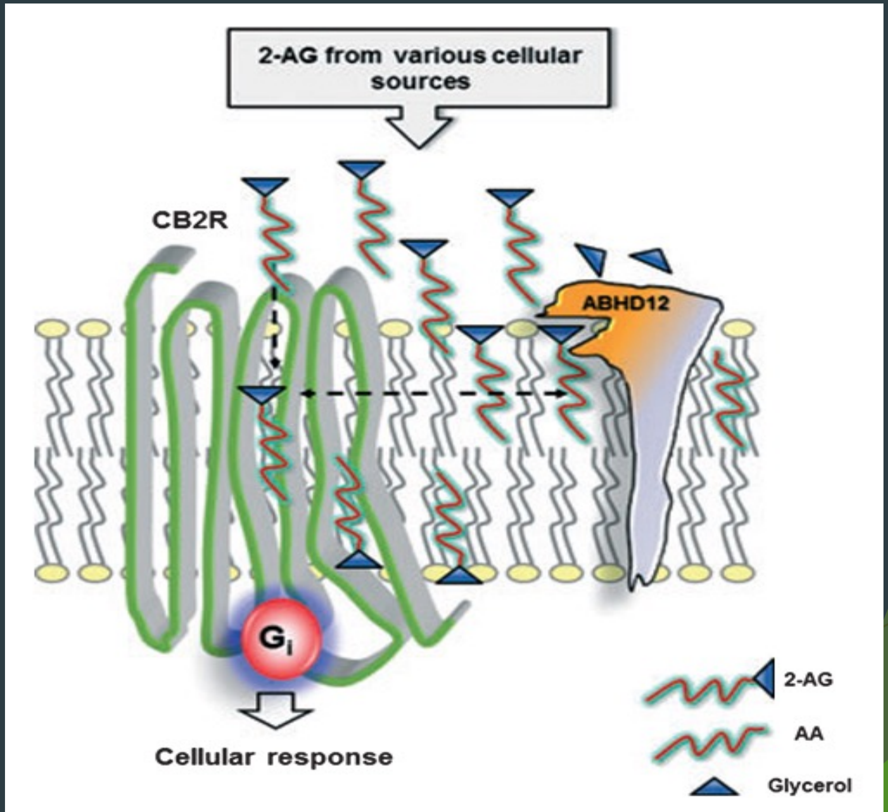


Savinainen et al. 2012 *Acta Physiol (Oxf).* 204:267

Metabolism of 2-AG *Alpha/Beta-Hydrolases*

- ABHD6 Clears Postsynaptic 2-AG at Site of Synthesis
- ABHD12 Clears Extracellular 2-AG





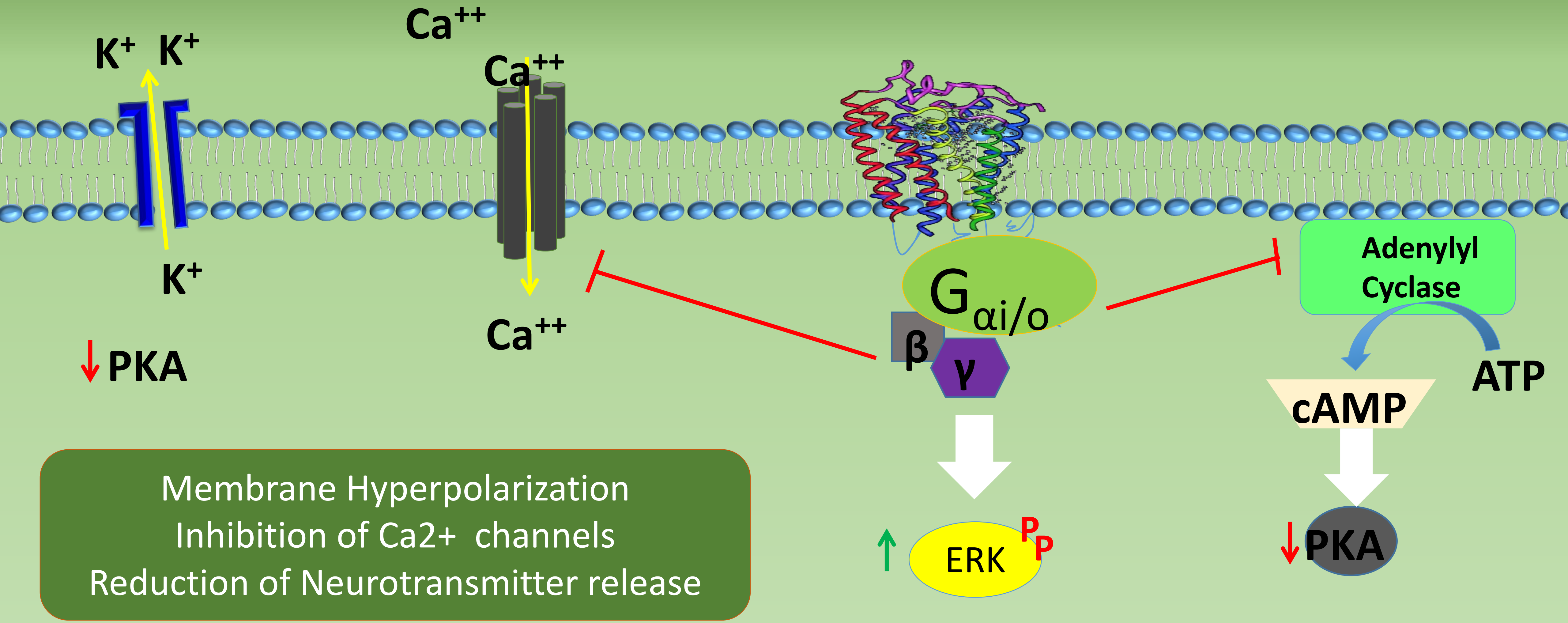
Savinainen et al. 2012 *Acta Physiol (Oxf).* 204:267

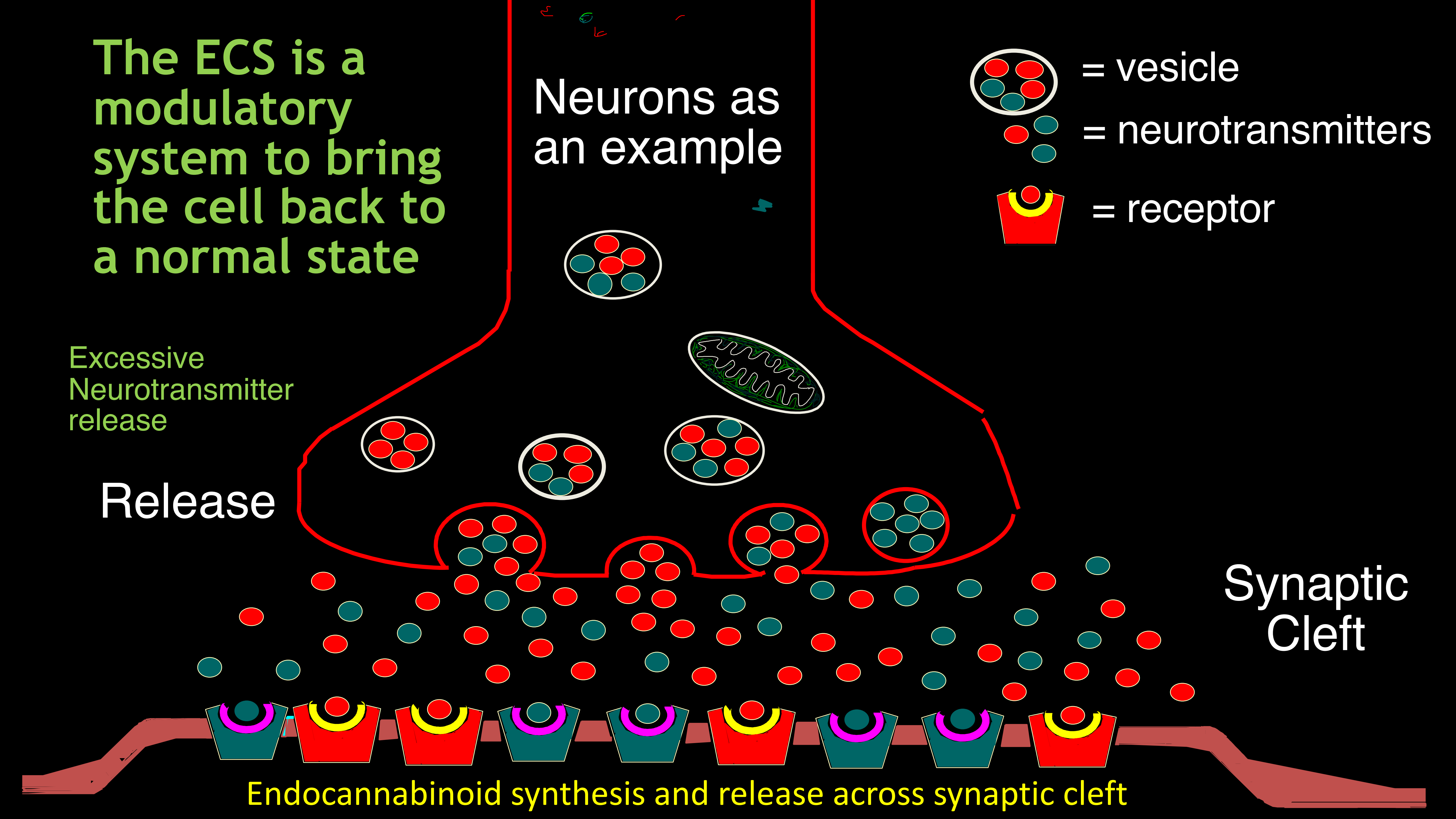
How does the ECS system serve a role as a modulatory system ?

Example

How the CB_1 receptor uses cellular signaling to regulate Neurotransmitter Release

Cellular Signaling from CB_1 Receptors




The ECS is a modulatory system to bring the cell back to a normal state
Neurons as an example
= vesicle
= neurotransmitters
= receptor
Excessive Neurotransmitter release
Release
Synaptic Cleft
Endocannabinoid synthesis and release across synaptic cleft

Receptors coupled to Gq proteins activate phospholipase C to convert membrane phospholipids to Diacylglycerol, a precursor of 2-Arachidonoylglycerol (2-AG)

Endocannabinoids are synthesized

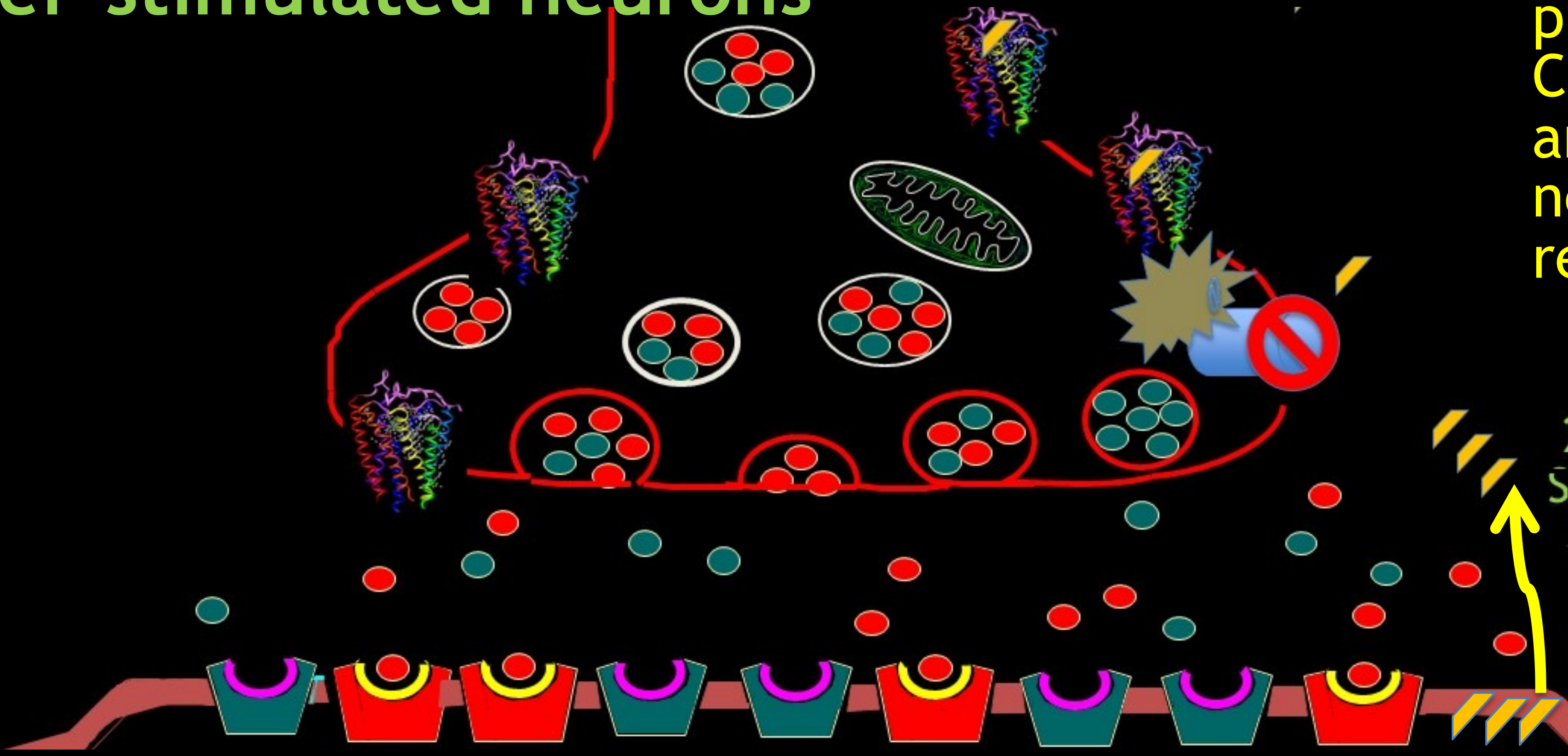
2-Arachidonoyl Glycerol (2-AG)

ArachidonylEthanolamide (Anandamide)

Endocannabinoids can diffuse out of the cell and across the synaptic cleft.

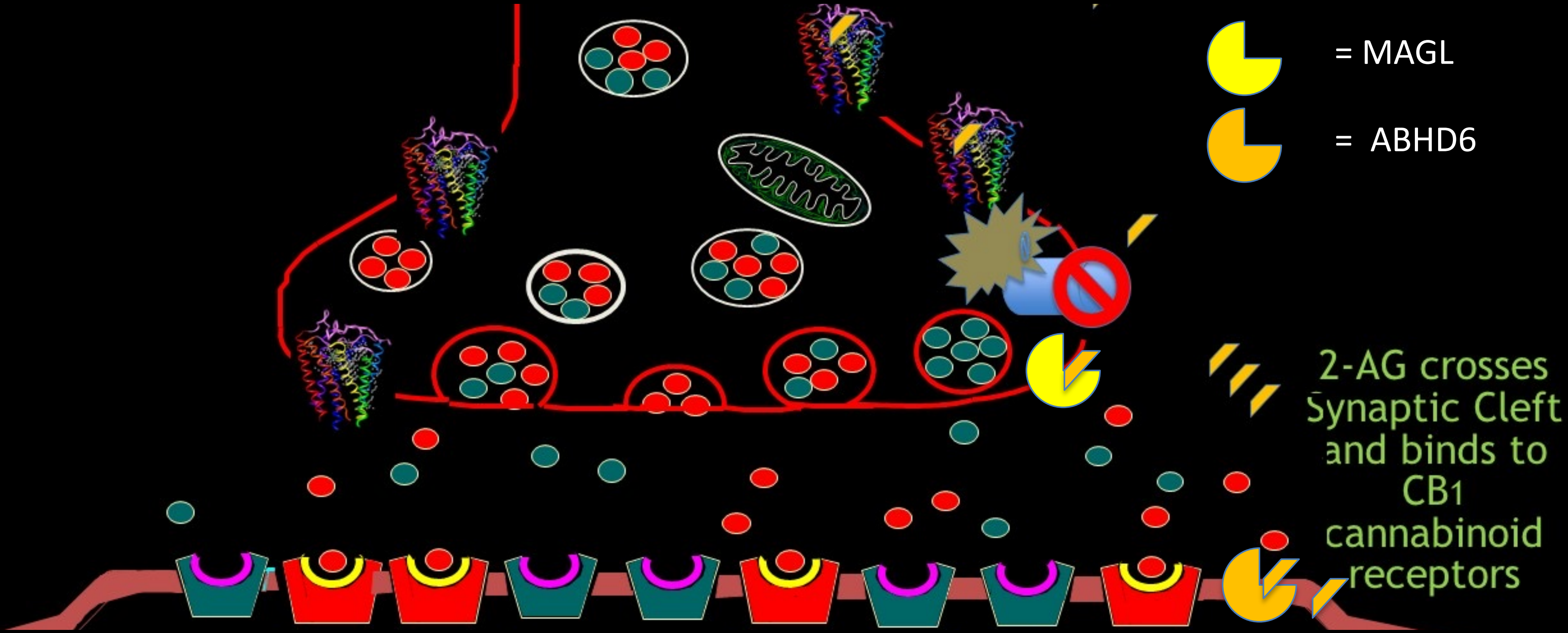
Endocannabinoids reduce neurotransmitter release in over-stimulated neurons

CB1 receptors couple to Gi proteins to block Ca2+ channels and reduce neurotransmitter release



2-AG crosses Synaptic Cleft and binds to CB1 cannabinoid receptors

2-AG Synthesis from Diacylglycerol by DAGLipase



2-AG is hydrolyzed by monoacylglycerol lipase (MAGL) at the presynaptic site or alpha-beta-hydrolase 6 (ABHD6) at its postsynaptic site of membrane production.

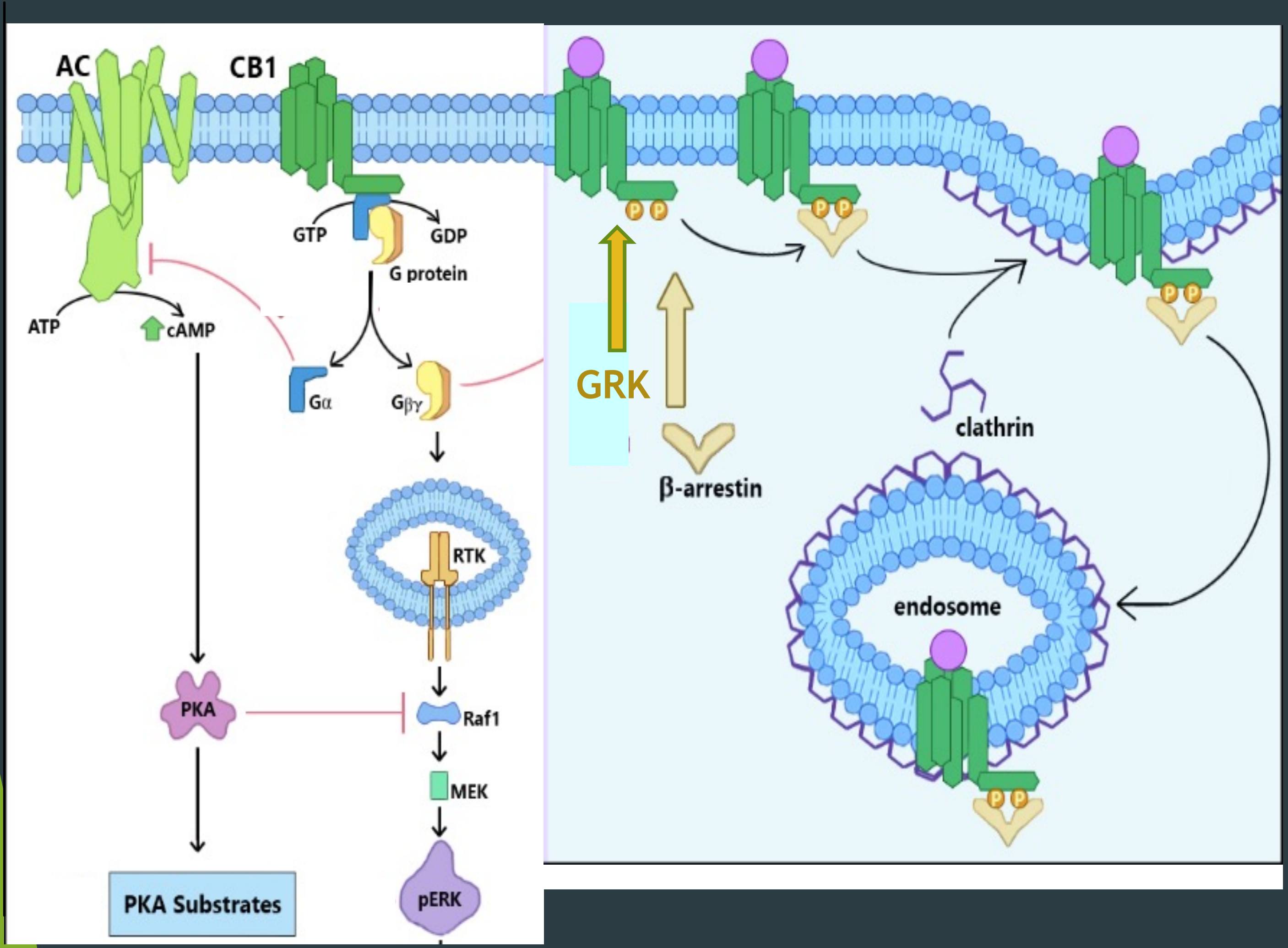
How does the ECS system serve a role as a modulatory system ?

How CB_1 receptor cellular signaling can define the outcomes in many different cell types:

Interaction with CB_1 receptor-associated proteins

Associated Proteins G-proteins; Beta-arrestins

Life Cycle of the G-protein Coupled Receptor



G-protein activation by the agonist-stimulated CB_1 Receptor causes dissociation of Gα from G$\beta\gamma$.

Gαi inhibits cAMP production which reduces phosphorylation of PKA substrate proteins.

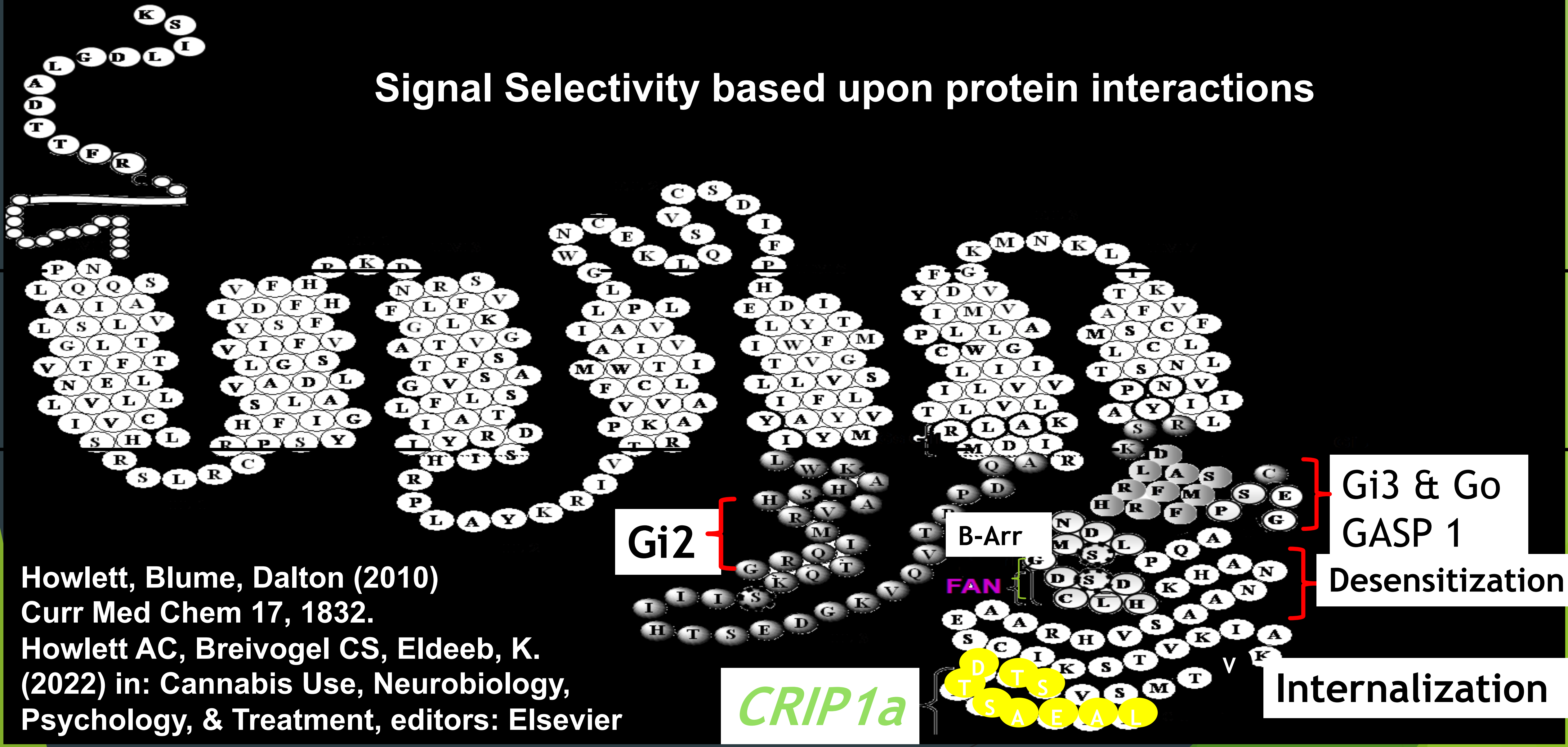
G-protein receptor kinase (GRK) phosphorylates agonist-CB_1 Receptor binds to β-arrestins 1 & 2.

This results in binding clathrin which causes internalization and formation of signaling endosomes.

Oliver et al 2020 Biomolecules

Diversity of CB_1 C-tail interactions

Signal Selectivity based upon protein interactions



Howlett, Blume, Dalton (2010) Curr Med Chem 17, 1832.
Howlett AC, Breivogel CS, Eldeeb, K. (2022) in: Cannabis Use, Neurobiology, Psychology, & Treatment, editors: Elsevier

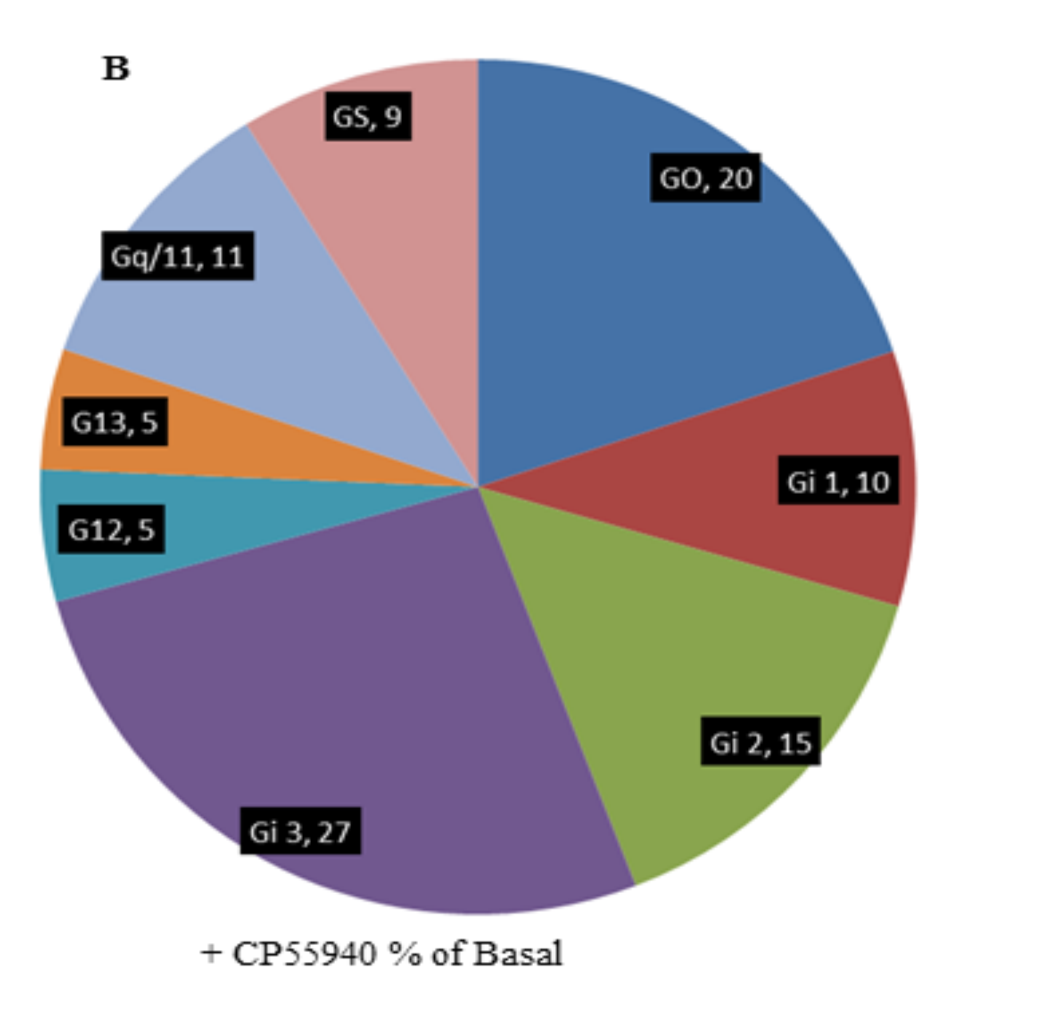
Agonist-stimulated CB1R can activate G-proteins, predominantly Gi/o

CP55940 full agonist stimulates CB1 Receptor G-protein activation:

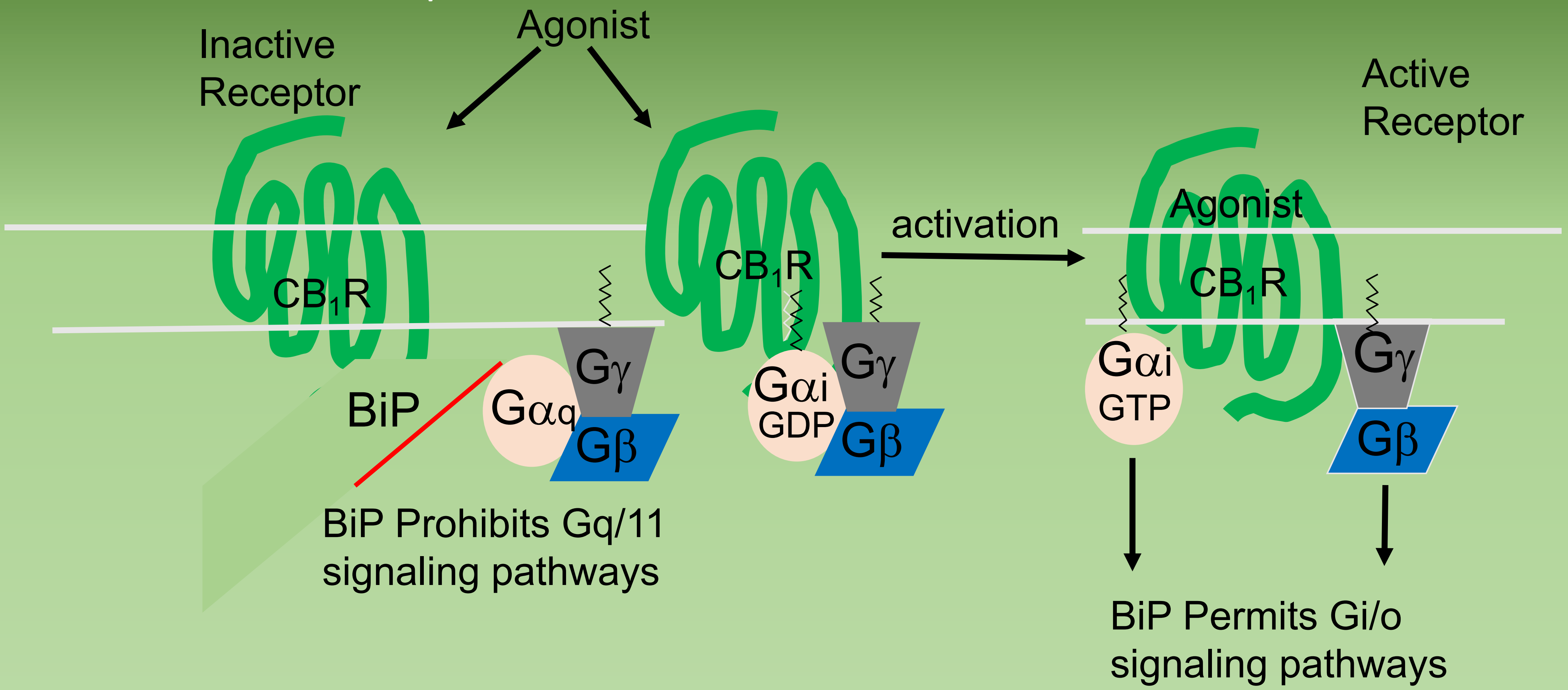
72% Gi/o proteins
11% Gq/11
10% G12/13
9% Gs

in N18TG1 cell model
GTPγS binding to G-proteins

Eldeeb et al., Meth Enz, 2017

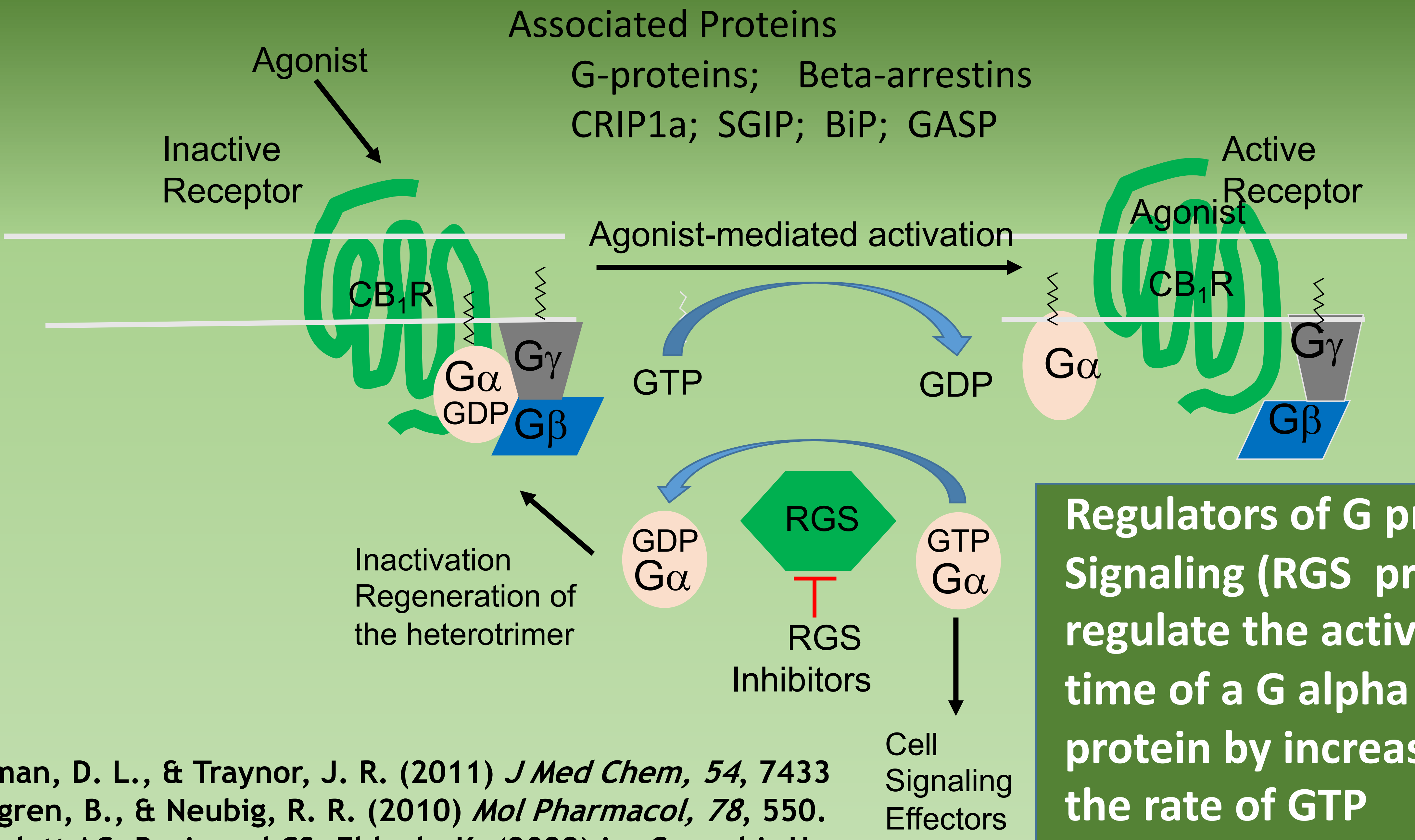


Associated Proteins: G-proteins; BiP



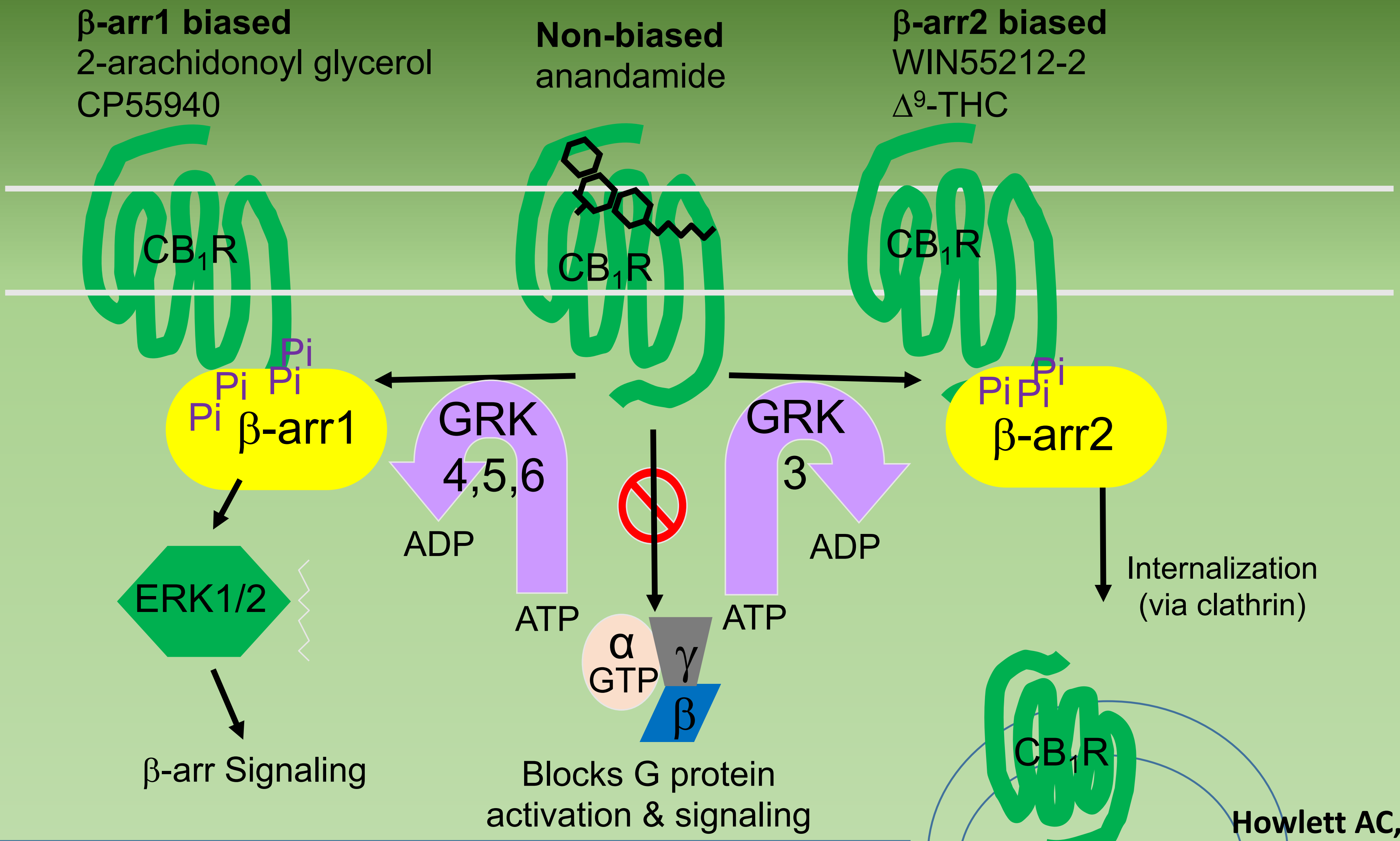
Costas-Insua … & Guzmán (2021)
***J Neurosci*, *41*, 7924**
Howlett AC, Breivogel CS, Eldeeb, K. (2022) in: Cannabis Use, Neurobiology, Psychology, & Treatment, Elsevier Inc.

Binding Protein (BiP), a member of the Heat Shock Protein 70 chaperone family, binds to CB_1 receptors to alter Ca^{2+}-mobilization signaling



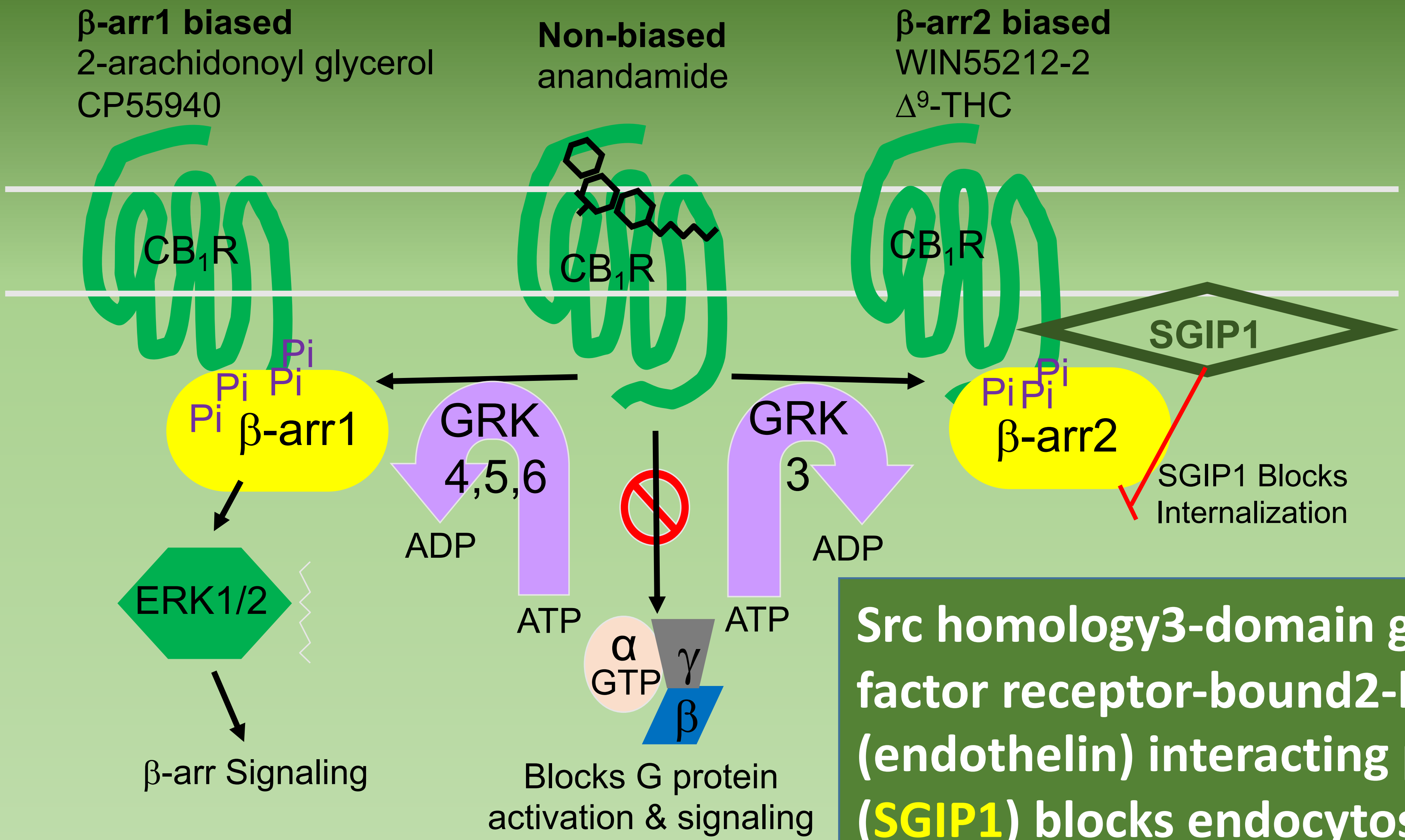
Regulators of G protein Signaling (RGS proteins) regulate the activation time of a G alpha protein by increasing the rate of GTP hydrolysis to GDP.

Roman, D. L., & Traynor, J. R. (2011) *J Med Chem, 54*, 7433
Sjogren, B., & Neubig, R. R. (2010) *Mol Pharmacol, 78*, 550.
Howlett AC, Breivogel CS, Eldeeb, K. (2022) in: Cannabis Use, Neurobiology, Psychology, & Treatment, Elsevier Inc.



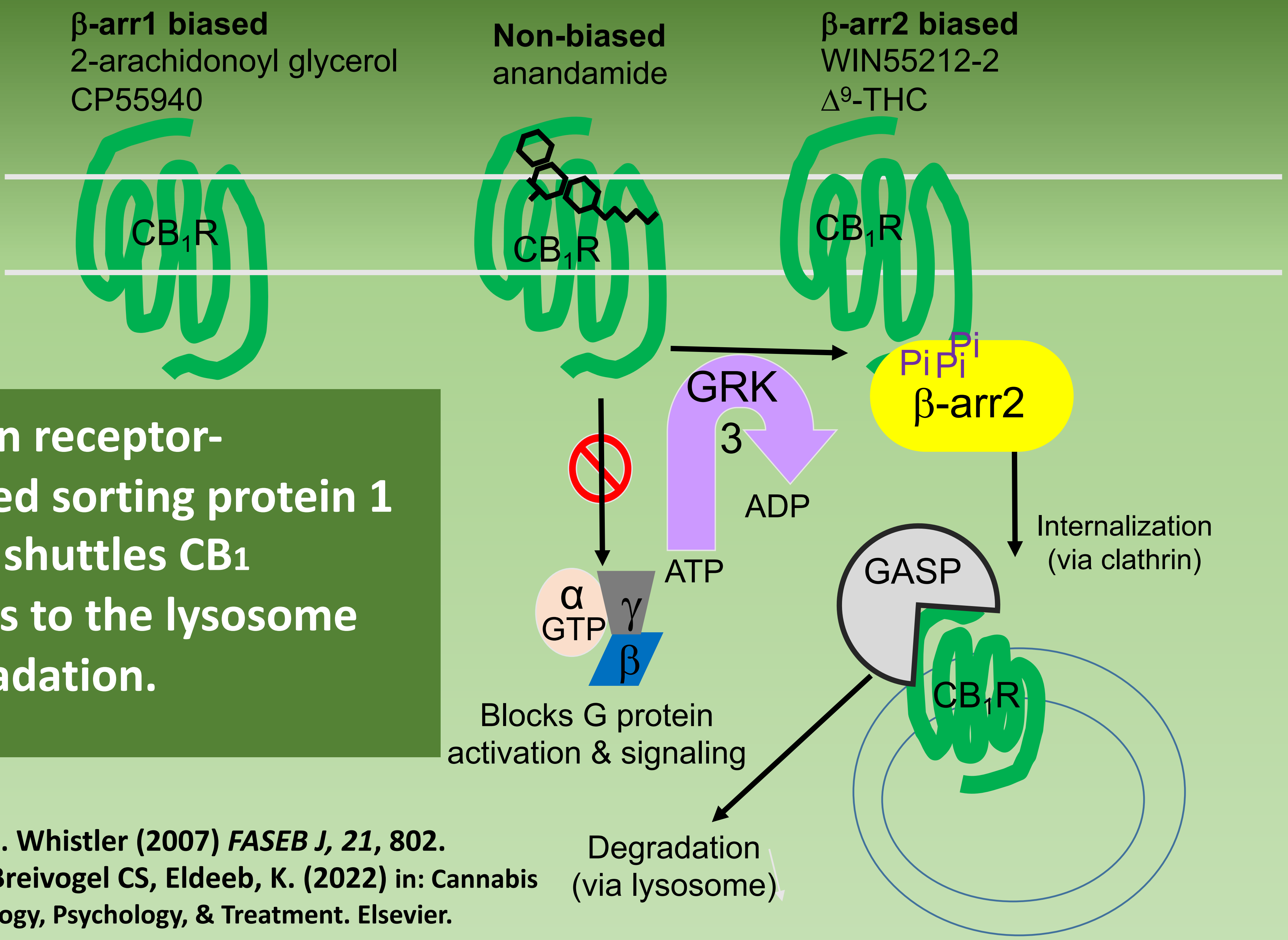
Phosphorylation of the CB_1 Receptor by G protein receptor kinases (GRKs) allows binding of β-arrestins 1 and 2.
β-Arrestins 1 and 2 initiate Extracellular Signal Regulated Kinases (ERK1/2) phosphorylation, or clathrin-mediated endocytosis, respectively.

Howlett AC, Breivogel CS, Eldeeb, K. (2022) in: Cannabis Use, Neurobiology, Psychology, & Treatment. Elsevier.



Howlett AC, Breivogel CS, Eldeeb, K. (2022) in: Cannabis Use, Neurobiology, Psychology, & Treatment, editors: Martin, Patel, Preedy. Elsevier Inc.

Src homology3-domain growth factor receptor-bound2-like (endothelin) interacting protein (SGIP1) blocks endocytosis of the CB_1 receptor-β-arrestin2 complex at the plasma membrane.

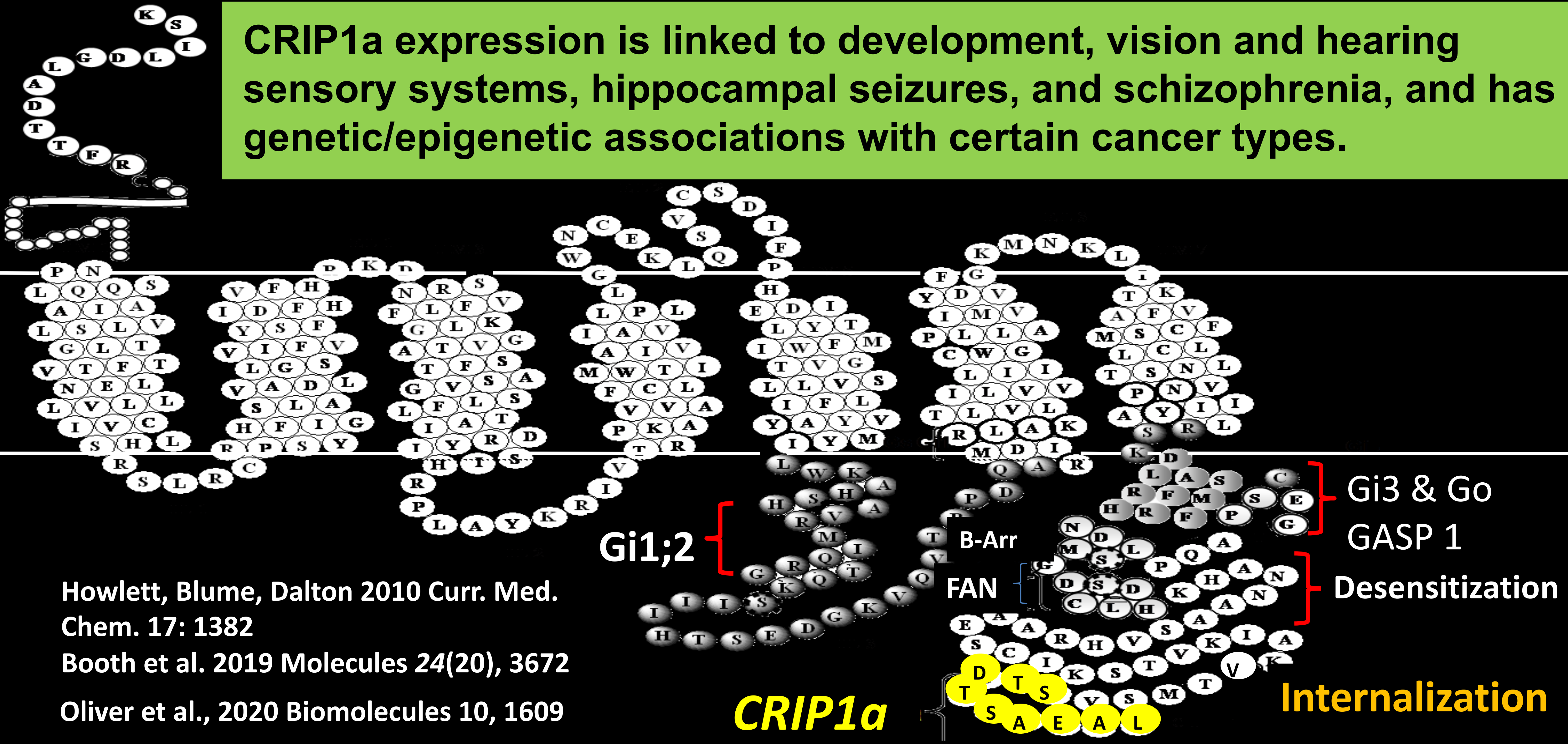


G-protein receptor-associated sorting protein 1 (GASP1) shuttles CB_1 receptors to the lysosome for degradation.

Martini et al… Whistler (2007) *FASEB J, 21*, 802.
Howlett AC, Breivogel CS, Eldeeb, K. (2022) in: Cannabis Use, Neurobiology, Psychology, & Treatment. Elsevier.

CB1 C-terminal interactions with regulatory proteins

CRIP1a expression is linked to development, vision and hearing sensory systems, hippocampal seizures, and schizophrenia, and has genetic/epigenetic associations with certain cancer types.

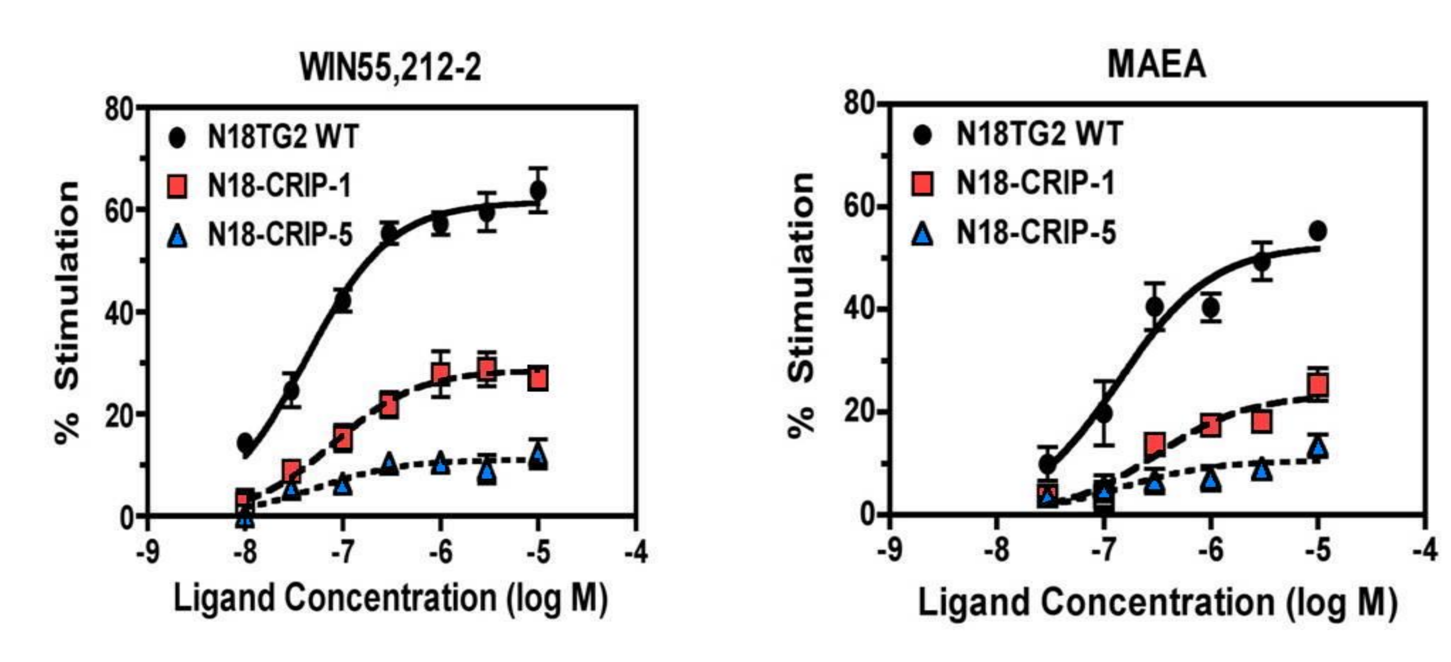


Howlett, Blume, Dalton 2010 Curr. Med. Chem. 17: 1382

Booth et al. 2019 Molecules *24*(20), 3672

Oliver et al., 2020 Biomolecules 10, 1609

CRIP1a reduces G protein activation by agonists

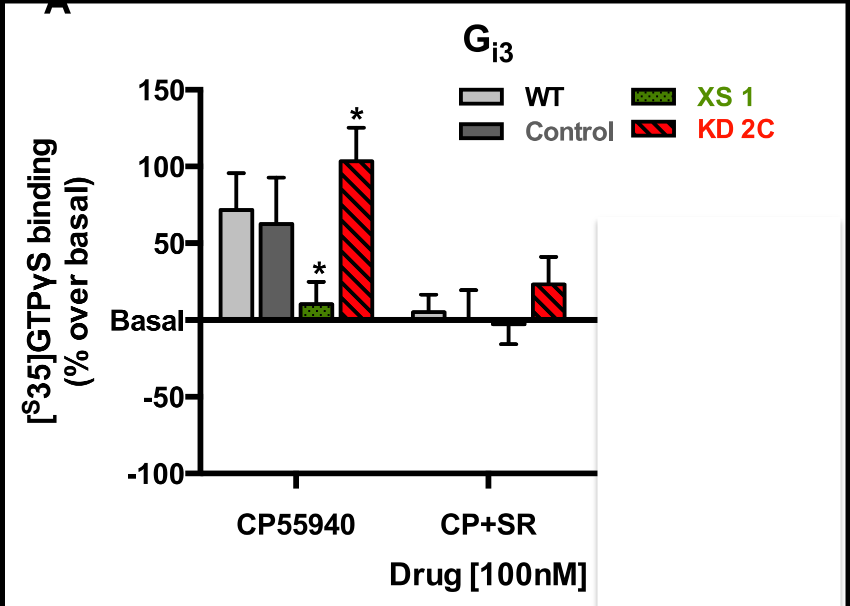


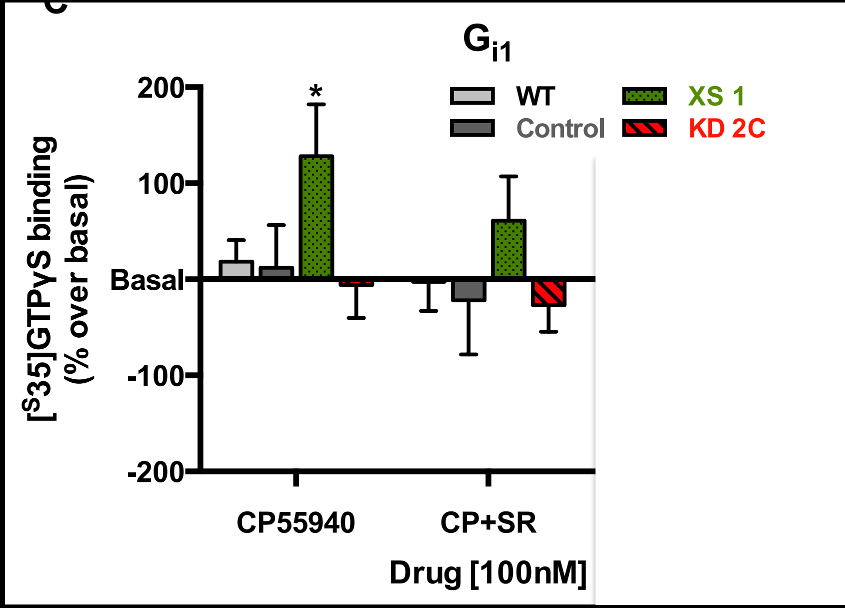
[^{35}S]GTPγS binding in N18TG2 or XS clones:

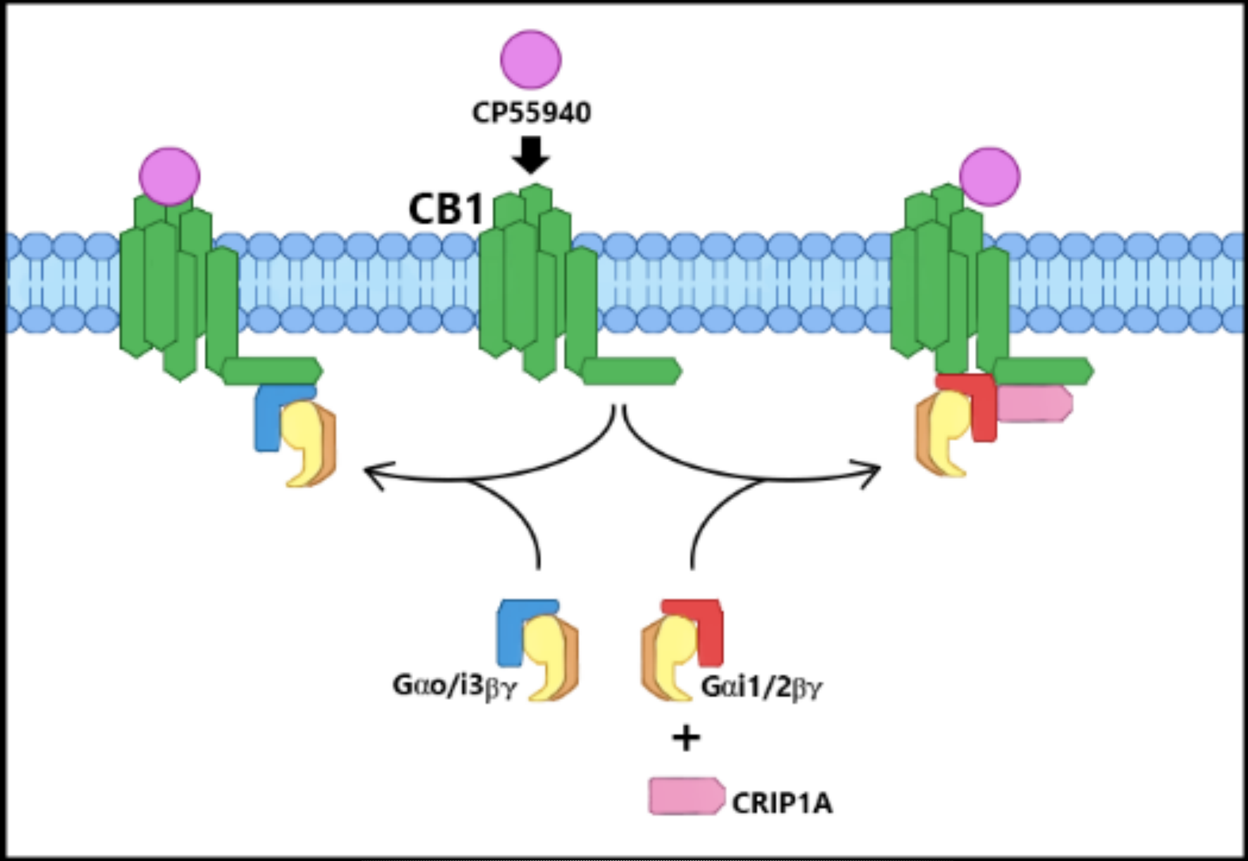
- WIN55212-2
- Methanandamide (mAEA)

Smith et al., 2015 Mol Pharmacol.

CRIP1a alters Gi/o subtype activation selectivity

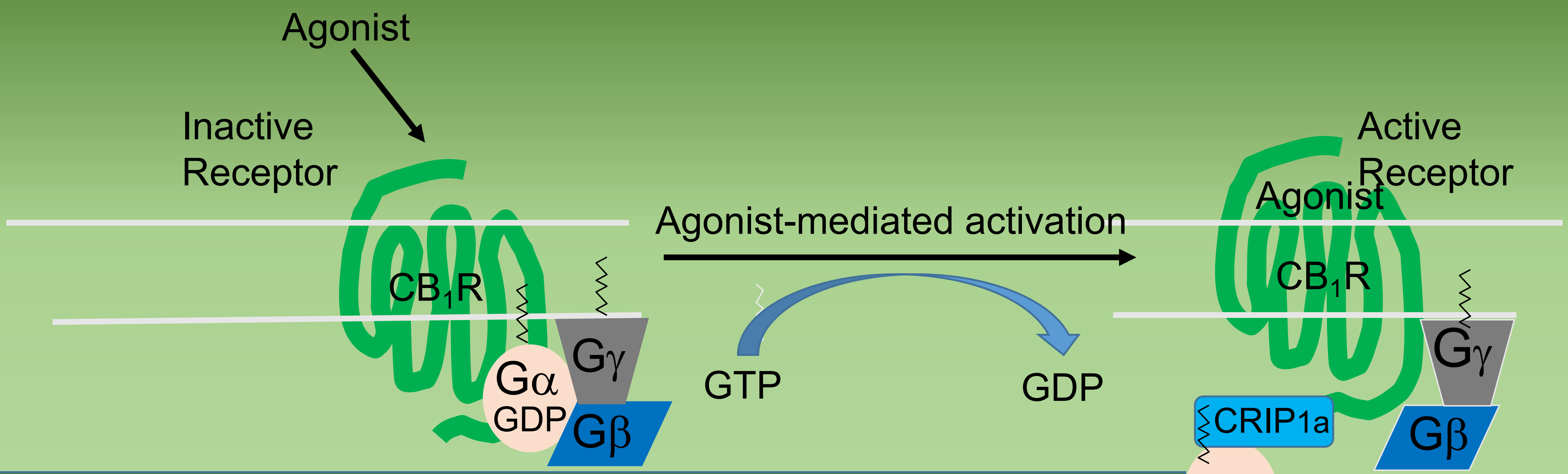




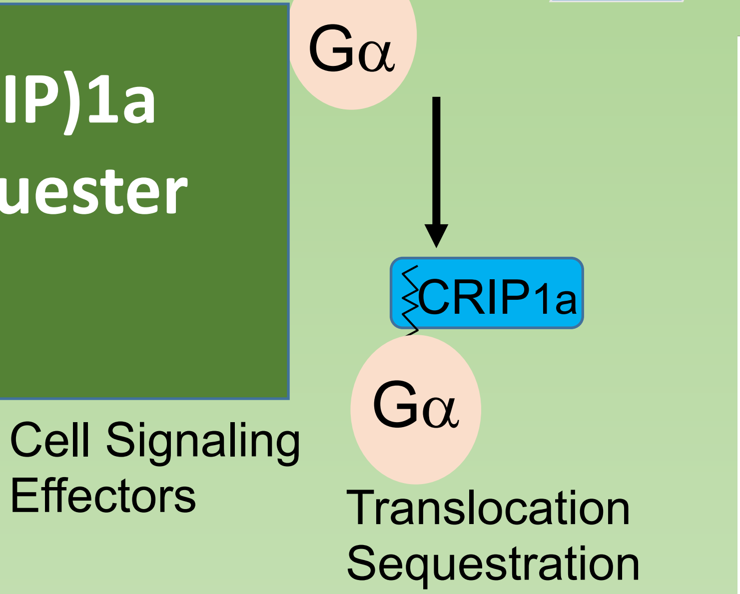


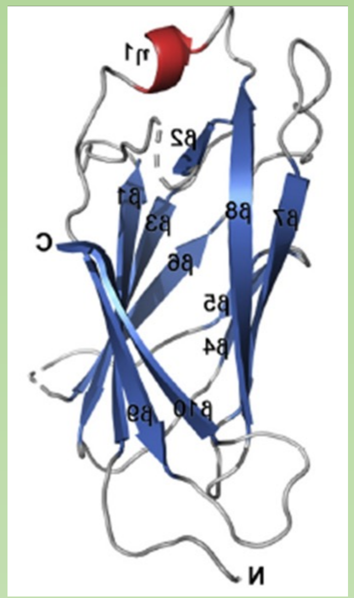
Blume et al. 2015 Cell. Signal. 27:716

Oliver et al., 2020 Biomolecules 10, 1609



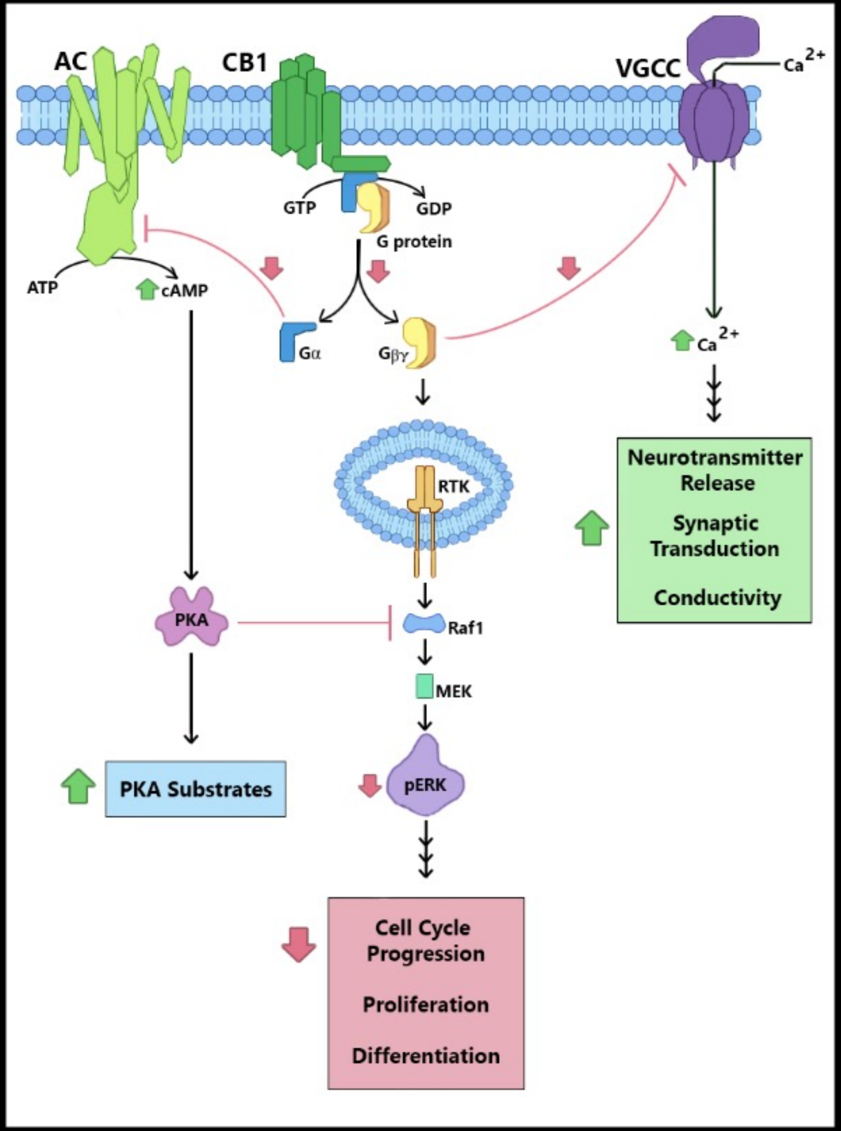
Cannabinoid Receptor Interacting Protein (CRIP)1a can bind to myristoylated Gαi proteins to sequester the Gαi or serve as a carrier within the cell.





Oliver et al., 2020 Biomolecules 10, 1609
Howlett AC, Breivogel CS, Eldeeb, K. (2022) in: Cannabis Use, Neurobiology, Psychology, & Treatment. Elsevier.

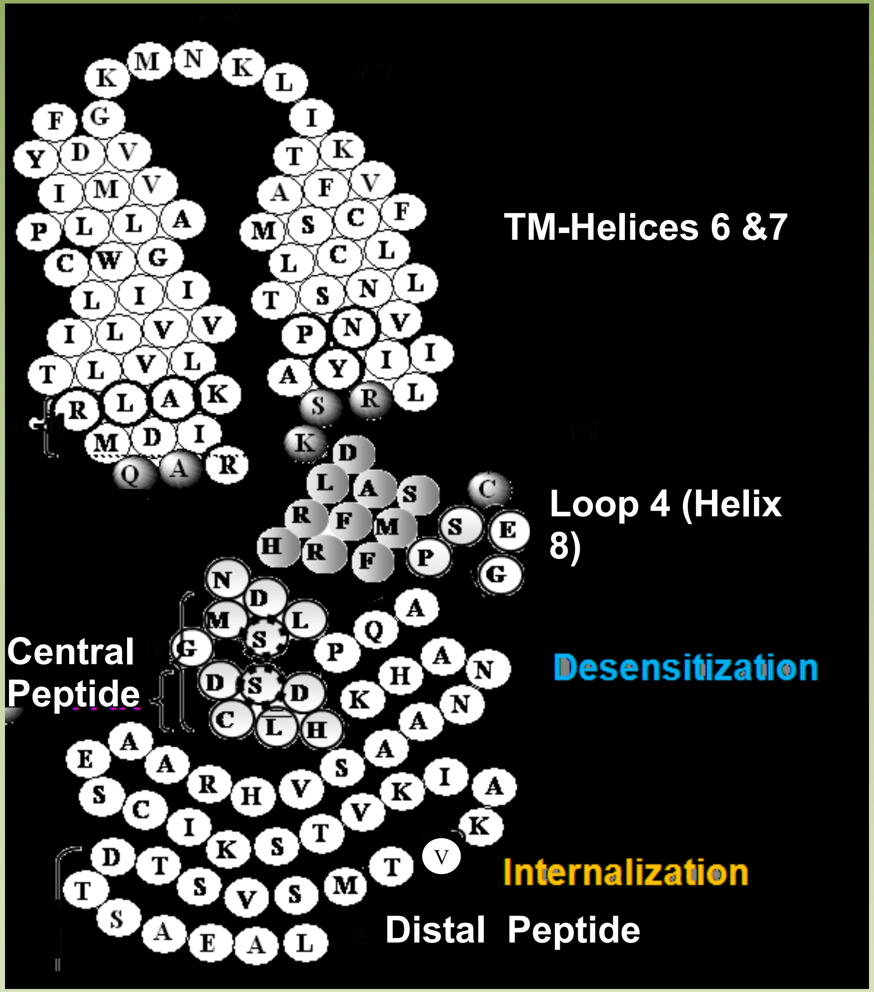
CRIP1a reduces sensitivity to Agonist-Stimulated Gi-inhibition of Adenylyl Cyclase and ERK phosphorylation.

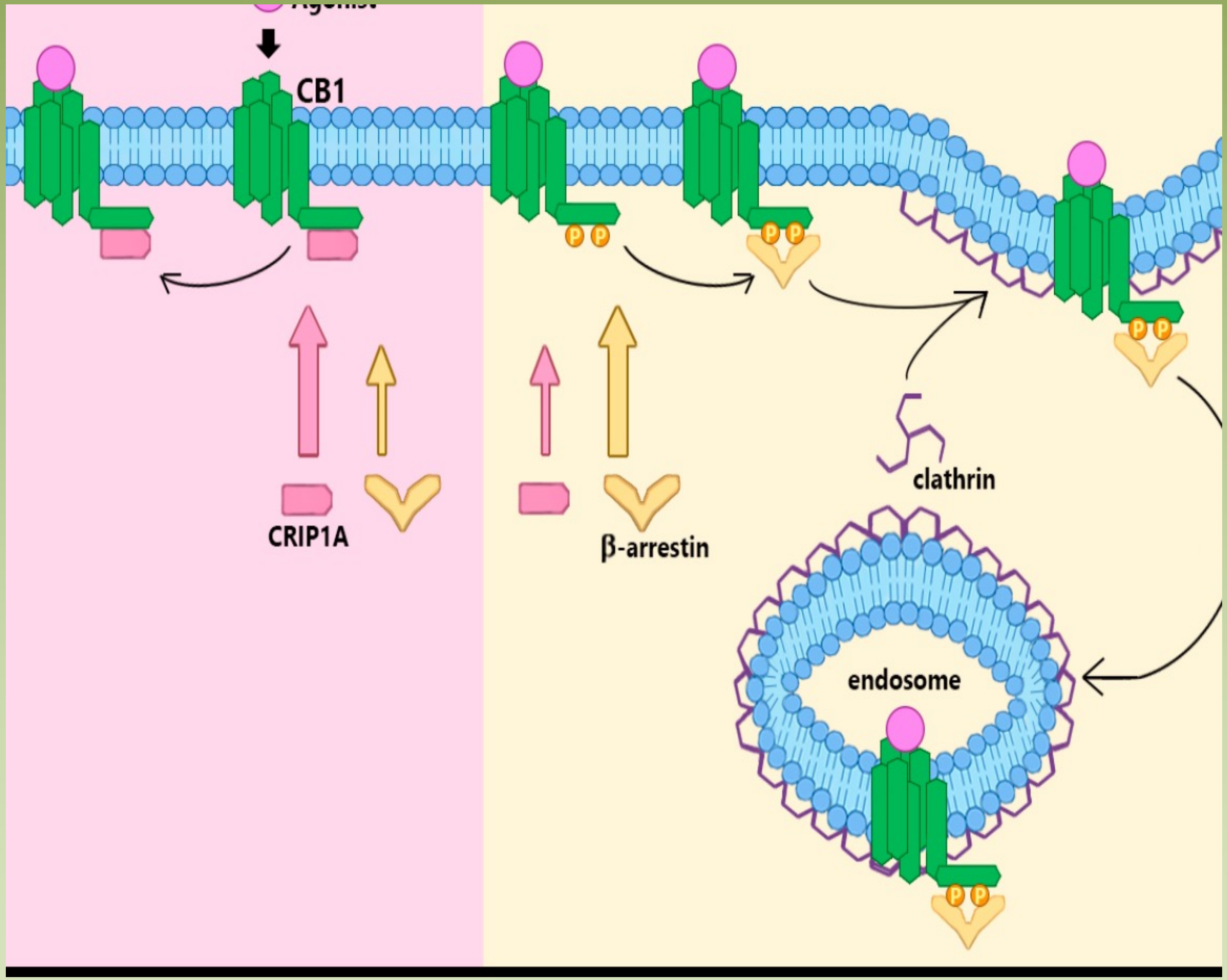


Blume et al. 2015 Cell. Signal. 27:716

Oliver et al., 2020 Biomolecules 10, 1609

CB1 Receptor Binding to CRIP1a interferes with binding to β-Arrestin 1 and 2





Blume et al. 2016 J. Neurochem. 139:396

Blume et al. 2017 Mol. Pharmacol. 91:75

Oliver et al., 2020 Biomolecules 10, 1609

The Endocannabinoid System Components

Summary

The Endocannabinoid System (ECS) Components

- CB_1 and CB_2 cannainoid Receptors
  - Agonists
  - Antagonists
  - Allosteric modulators
- Endocannabinoids: Anandamide, 2-AG
- Endocannabinoid synthesis enzymes
  - N-acylphosphatidylethanolamine Phospholipase D (NAPE-PLD)
  - Diacylglycerol lipase (DAGL)
- Endocannabinoid degradation enzymes
  - Fatty acid amide hydrolase (FAAH)
  - Monoacylglycerol lipase (MAGL)
  - ABHD6, ABHD12

Summary

The Cannabinoid Receptor Interactome

- Cellular Signaling to Activate Responses
  - Cellular Signaling in the ECS
  - Receptor-Associated Proteins
    - G-proteins
    - Beta-arrestins
    - CRIP1a
    - SGIP
    - BiP
    - GASP

Conclusions

Organization of the ECS enzymes, Cannabinoid Receptors, and interactome proteins in different cell types can define the stimulus & response.

Predictions:

- Cellular Responses to exogenous Δ9-THC will differ in different cell types depending upon receptors and interactome proteins expressed.
- Δ9-THC alone may act as an agonist.
- Δ9-THC may act as an antagonist against full agonist 2-AG.
- CBD is not an agonist at CB_1 Receptors, but might allosterically reduce the response to 2-AG or Δ9-THC.
- CBD and minor phytocannabinoids probably act by non-ECS mechanisms.