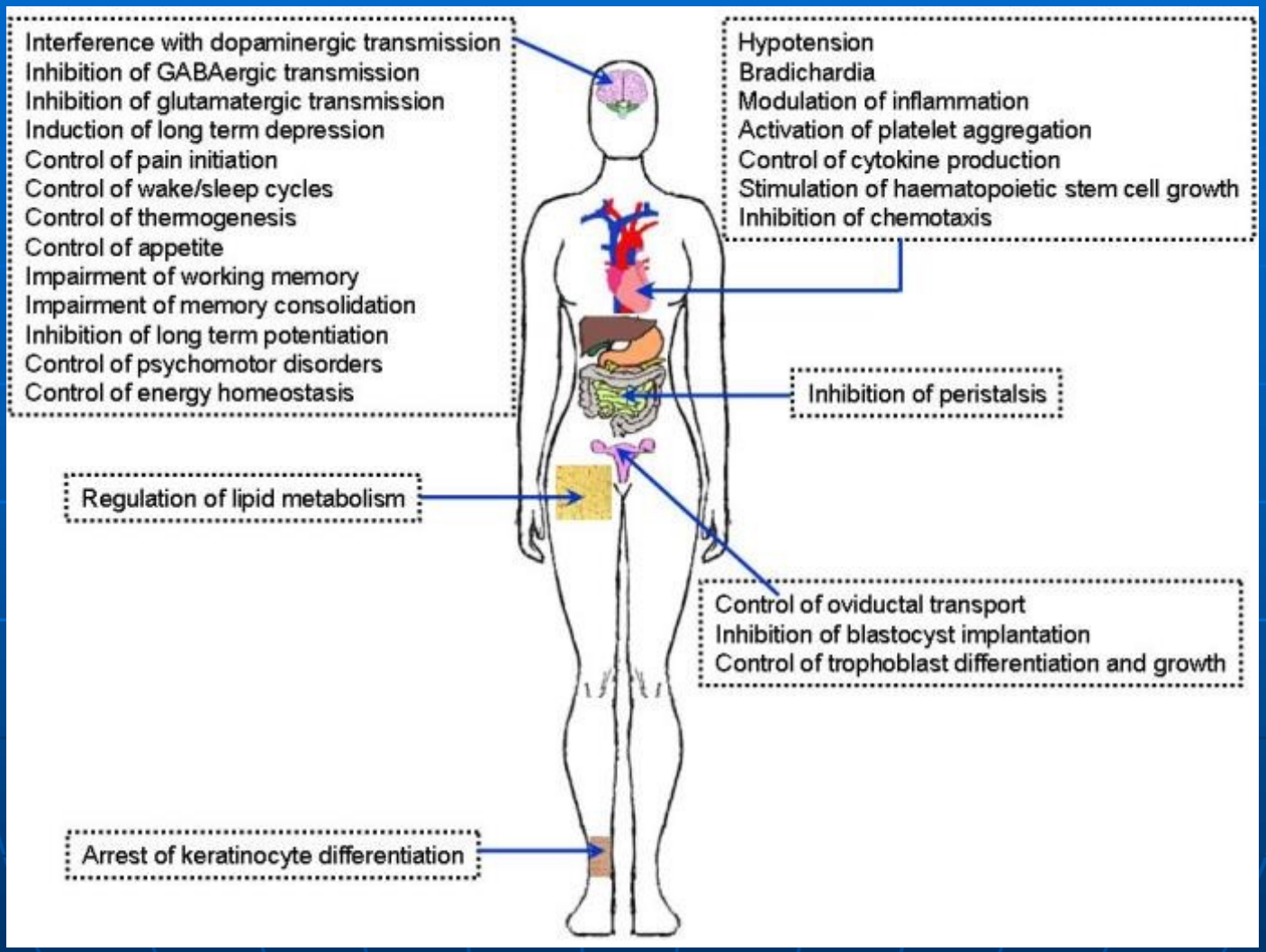
Nutritional Role of endocannabinoids



From Maccarrone & Dainese, et al., Annual Review of Nutrition, (2010).

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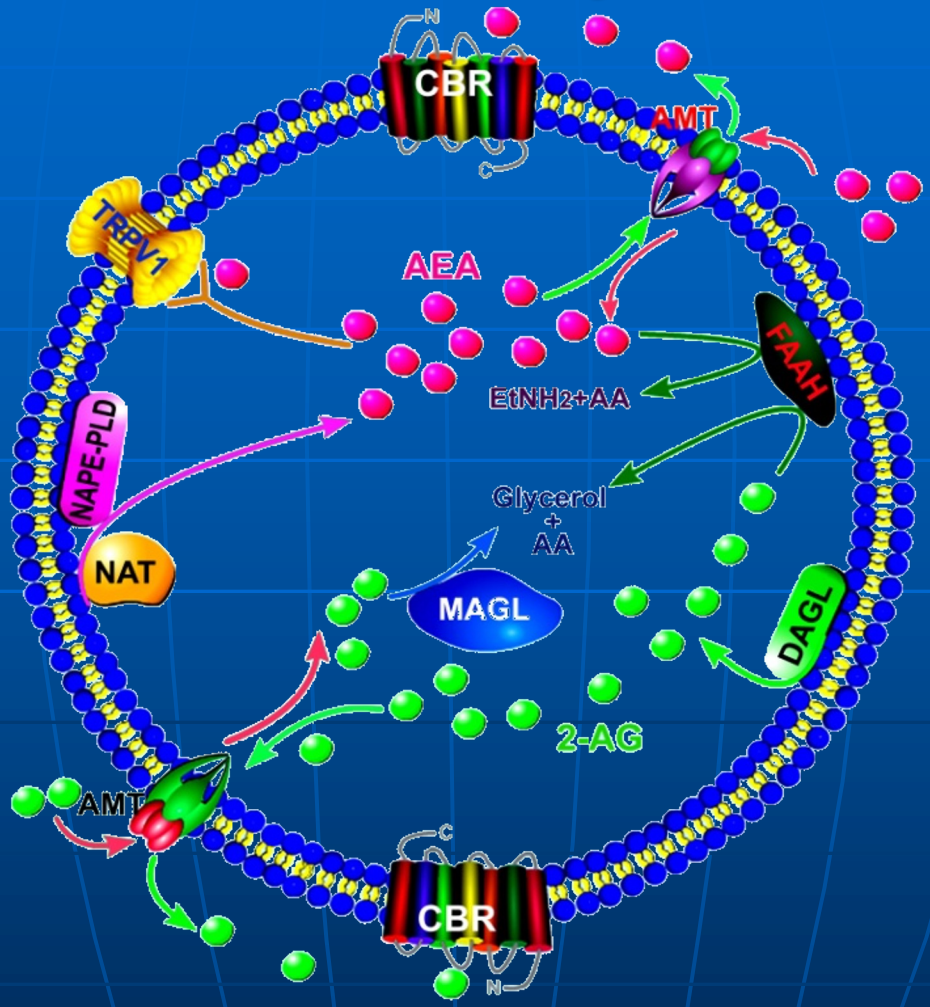
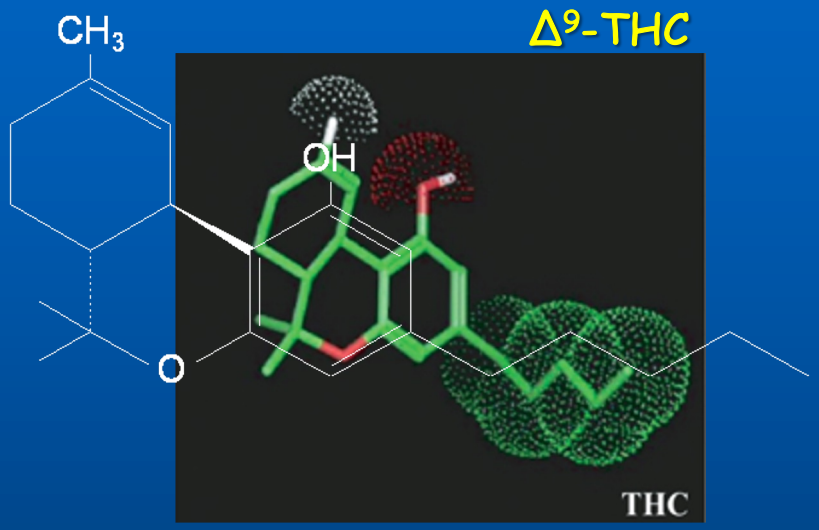
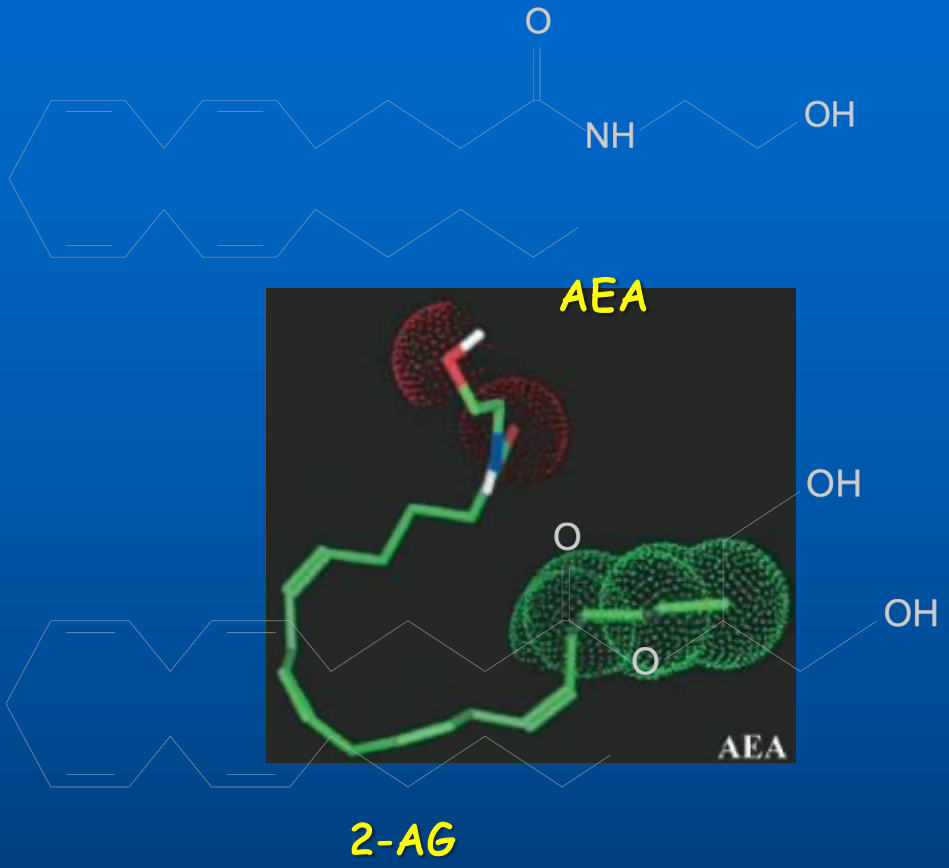
The endocannabinoid system and n-3 fatty acids



The endocannabinoids



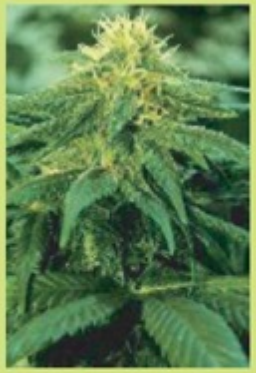
Δ9-THC

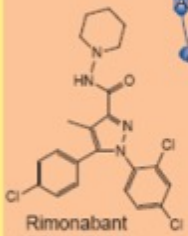


AEA

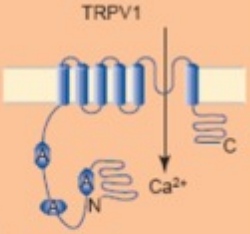
2-AG

The history of the (endo)cannabinoids





Rimonabant (SR141716A)



200
The therapeutic properties of cannabis are described in Chinese pharmacopoeia

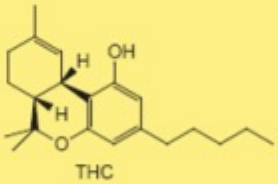
1838–1840
Sir W.B. O'Shaughnessy assesses methodically the medicinal properties of cannabis

Wood *et al.* isolate cannabinol from cannabis resin
1899

1932
Cahn elucidates part of the structure of cannabinol

Todd *et al.* (and Adams *et al.* in the USA) fully elucidate and synthesize cannabinol
1940

1964
Gaoni and Mechoulam elucidate the structure of THC



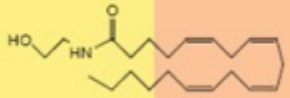
THC

Cannabinoid pharmacology is thoroughly investigated
1970–1990

1988
Howlett's group identifies specific THC binding sites in the brain

Matsuda *et al.* clone the CB_1 receptor
1990

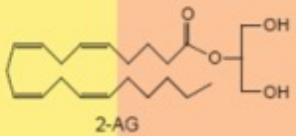
1992
Mechoulam's group in collaboration with Pertwee's group identify the first endocannabinoid, anandamide



Anandamide

Munro *et al.* clone the CB_2 receptor
1993

1995
Mechoulam's group and Waku's group identify the second endocannabinoid, 2-AG



2-AG

Rinaldi-Carmona *et al.* at Sanofi develop the first CB_1 receptor antagonist
1994

1998
House of Lord's report on medical cannabis;
Di Marzo *et al.* propose the existence of interactions between endocannabinoids and vanilloid receptors

Cravatt *et al.* clone the first endocannabinoid-degrading enzyme, FAAH
1996

2003
Bisogno *et al.* clone the first endocannabinoid-biosynthesizing enzymes

Zygmunt *et al.* and Smart *et al.* show that anandamide activates vanilloid receptors
1999–2000

????
Cloning of new cannabinoid receptors; identification of other endocannabinoid enzymes; cloning of the endocannabinoid transporter; more endocannabinoid-based therapies

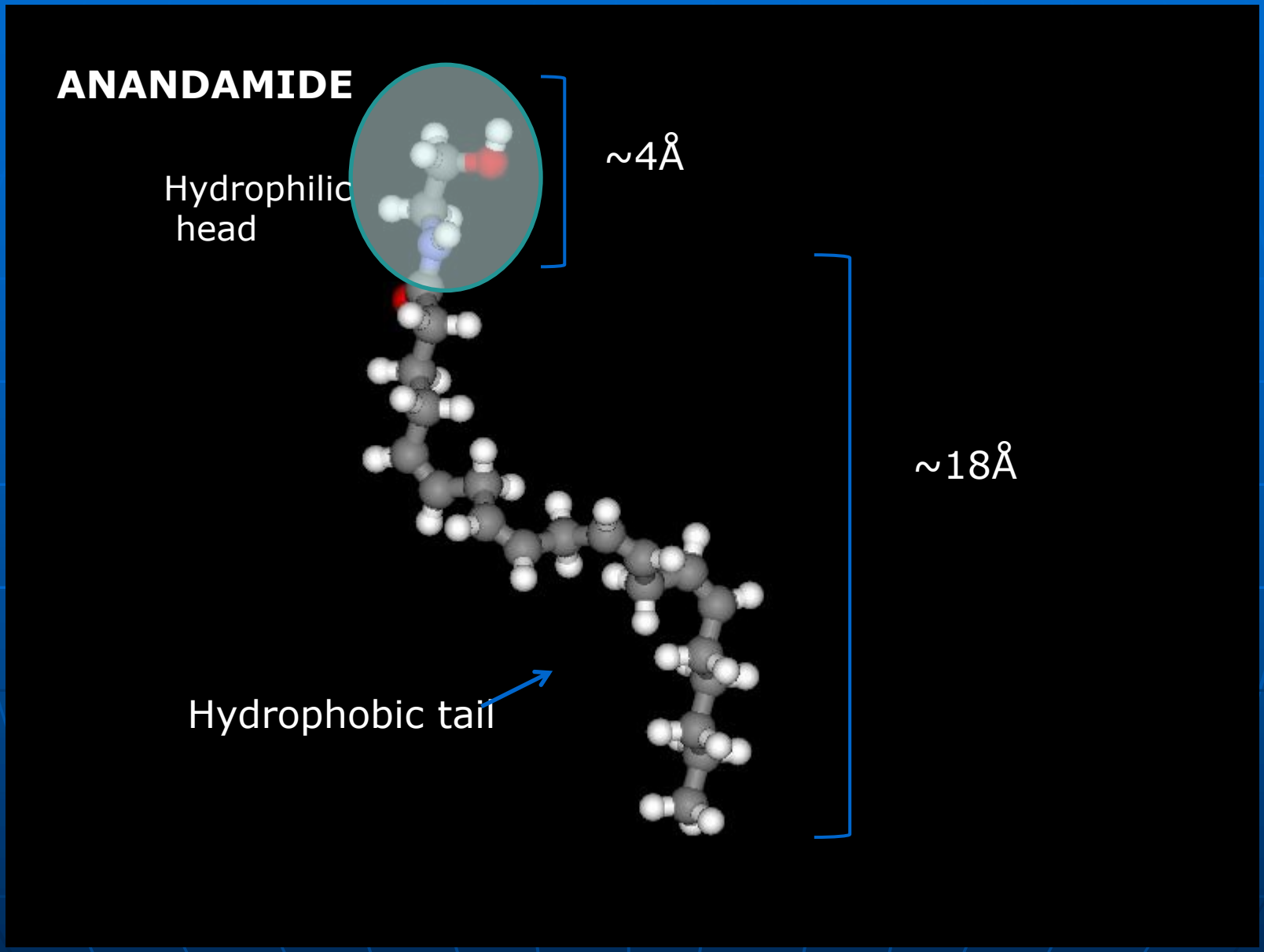
Sativex ® approved for sale in Canada; regulatory approval is filed to sell rimonabant in the USA; the Aberdeen group discovers an allosteric site on CB_1 receptors
2005

Cannabis research

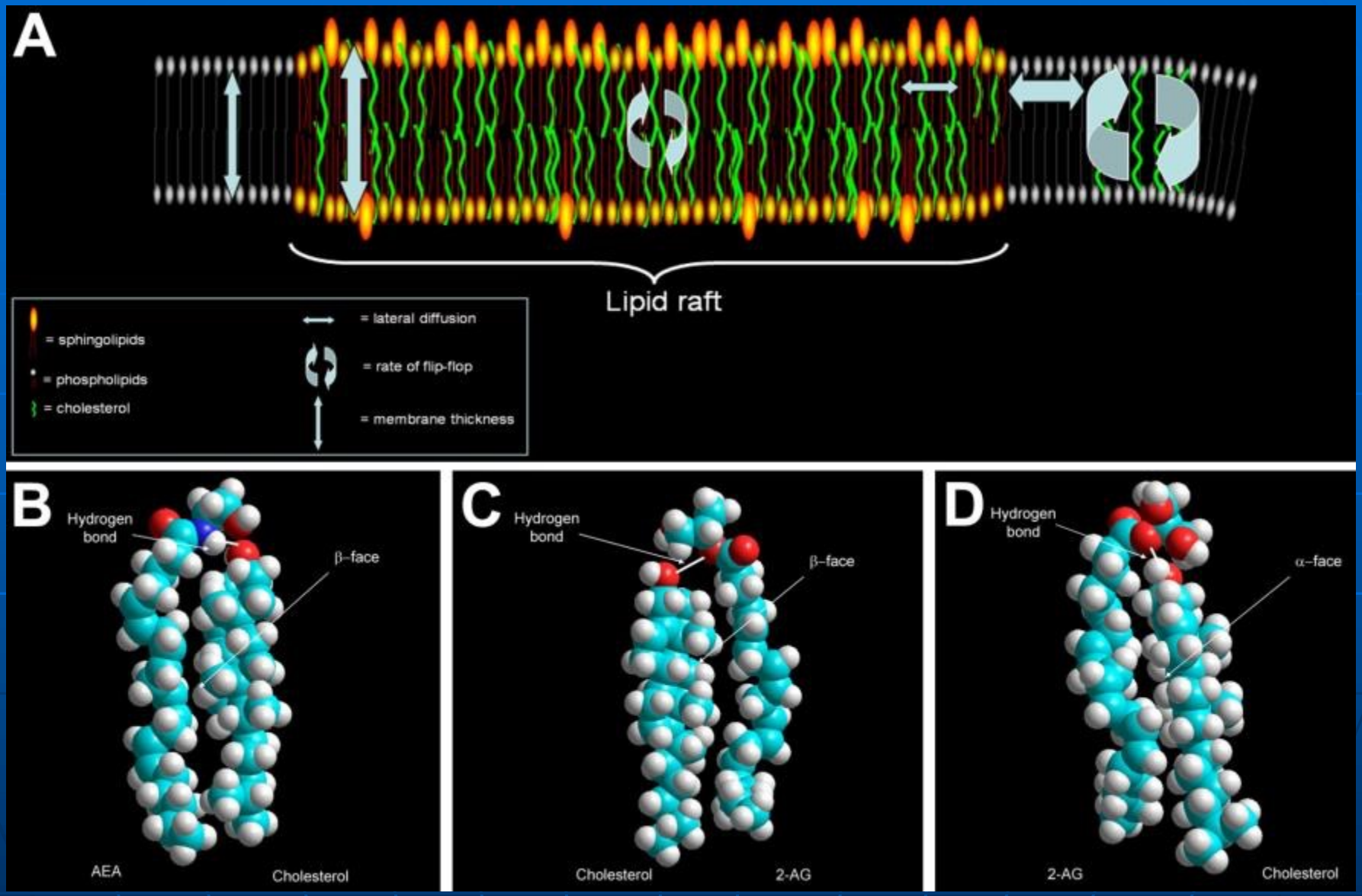
Cannabinoid research

Endocannabinoid research

Di Marzo, 2006


ANANDAMIDE
Hydrophilic head
~4Å
~18Å
Hydrophobic tail

Specific lipid-lipid interaction modulating the diffusion and transport of endocannabinoids



Dainese E, Oddi S, Bari M, Maccarrone M. Modulation of the endocannabinoid system by lipid rafts. Curr Med Chem. 2007;14(25):2702-15.

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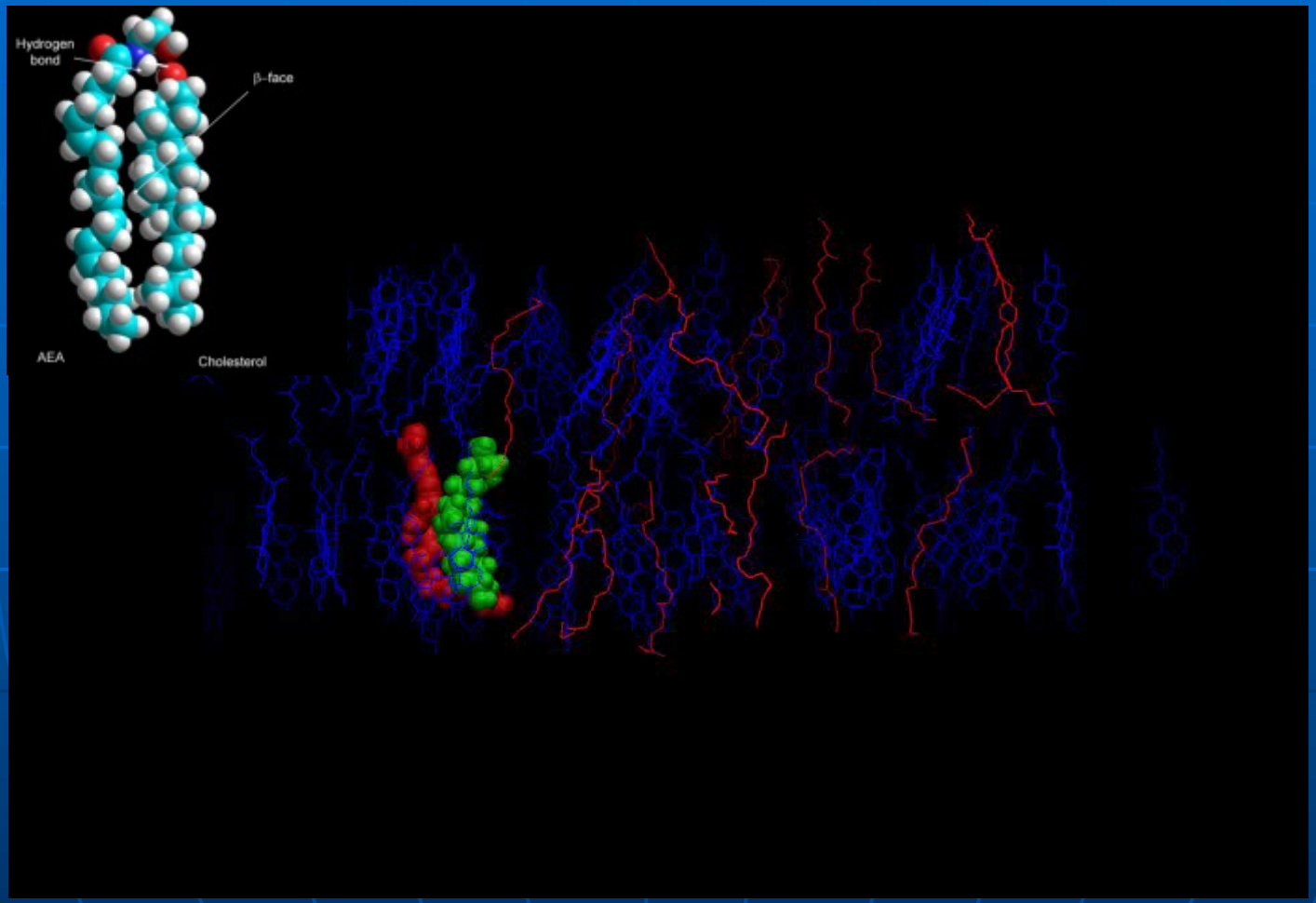
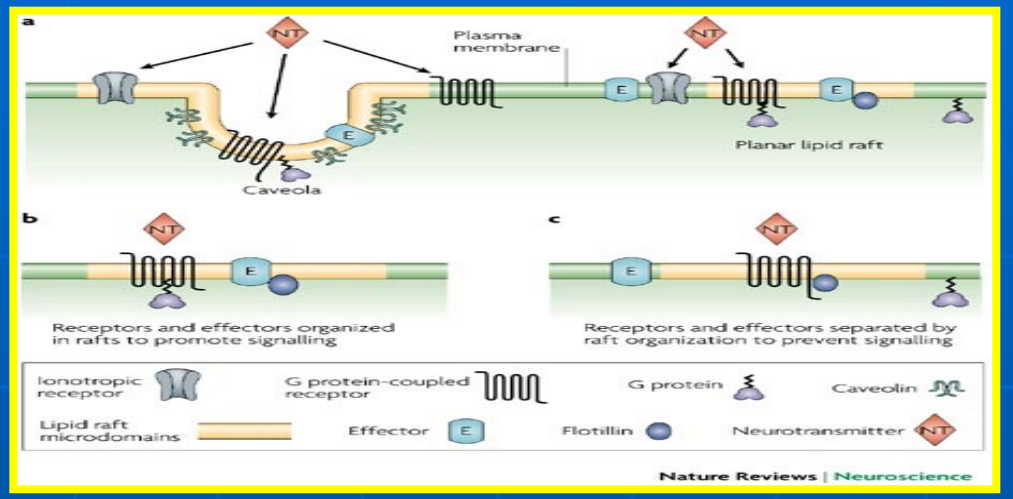
MD shows that the presence of cholesterol enhance the flip-flop rate of AEA



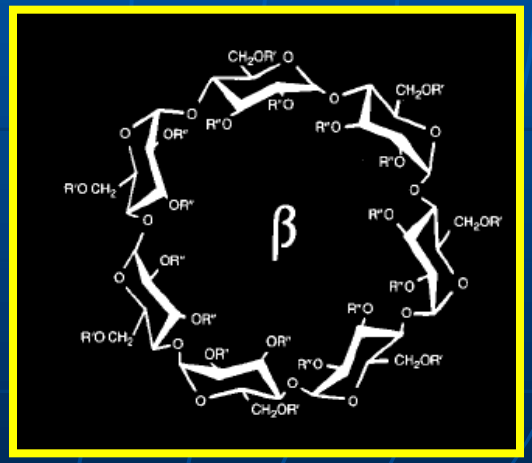
LRs are an ideal platform for GPCRs signaling

Table 1 | Examples of G-protein-coupled receptors that localize in lipid raft/caveolae before ('pre-agonist') and/or after ('post-agonist') treatment with agonists

	Pre-agonist	Post-agonist
Endothelin (ETA and ETB)	+	+
Cholecystokinin (CCK)		+
Muscarinic cholinergic	+	+
Bradykinin (B_1 and B_2)	+	+
Lysophosphatidic acid (LPA-1)	+	
Angiotensin II (AT-1)		+
β_1- and β_2-adrenergic	+	
P_2Y (P_2Y_1)	+	
Adenosine A1	+	+
Sphingosine 1-phosphate (EDG-1)	+	+
Smoothened/patched	+	
Serotonin ($5HT_{2A}$)	+	
Calcium-sensitive	+	
α_1-Adrenergic (α_{1B})	+	
Chemokine CCR_2		+
Metabotropic glutamate (mGlu1)	+	
Gonadotrophin-releasing hormone (GnRH)		+
Oxytocin	+	
Growth-hormone releasing hormone		+
Dopamine [D_1; D(1A)]	+	+
Neurokinin 1	+	
μ-Opioid receptor	+	



(Allen *et al.*, 2007)



(MCD)

Modulation of ECS by membranes
Effect of plasma membrane cholesterol on CB receptors function

Receptor	Treatment			
	Cholesterol depletion		Cholesterol enrichment	
	Binding	*Signaling*	*Binding*	*Signaling*
CB1R	↑	↑	↓	↓
CB2R	↔	↔	↔	↔
β_2-AR	↑	↑	↓	↓
Serotonin$_{1A}$R	↑	↑	↓ or ↔	↓ or ↔

Current Medicinal Chemistry, 2010, 17, 1487-1499 1487
Interaction of Endocannabinoid Receptors with Biological Membranes
E. Dainese, S. Oddi and M. Maccarrone

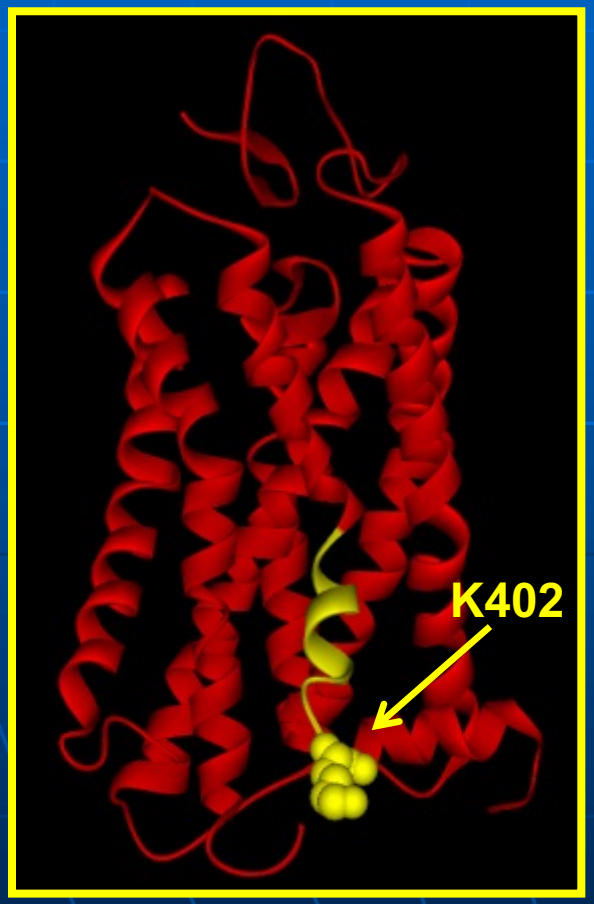
Putative cholesterol binding sites in CBRs: Cholesterol Recognition Aminoacid Consensus (CRAC)

CB2R	NSMVNPVIYALRSGEIRSSAHHCLAHWKKCVR	322
CB1R	NSTVNPIIYALRSKDLRHAFRSMFPSCEGTAQ	420
β_2AR	NSGFNPLIYC-RSPD----FRIAFQELLCLRR	346
SerR	NSLLNPVIYAYFNKD----FQNAFKKIIKCKF	417

CRAC seq: V/L-X_{1-4}-Y-X_{1-4}-K/R

Transmembrane helix 7

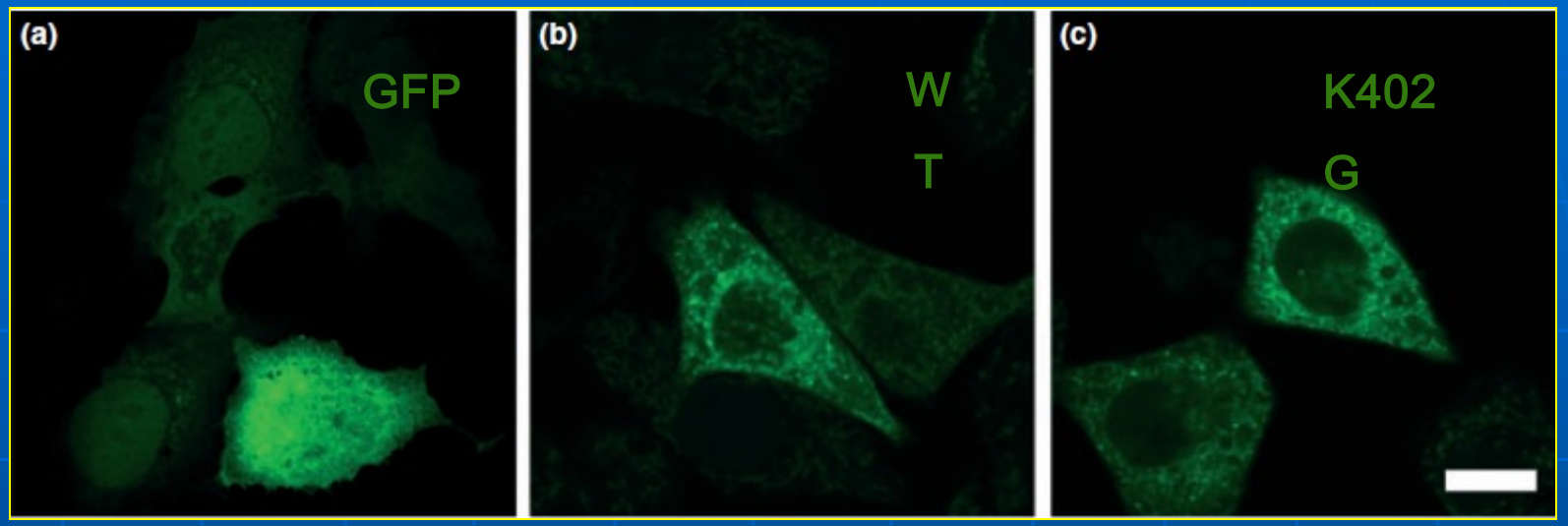
CB1R

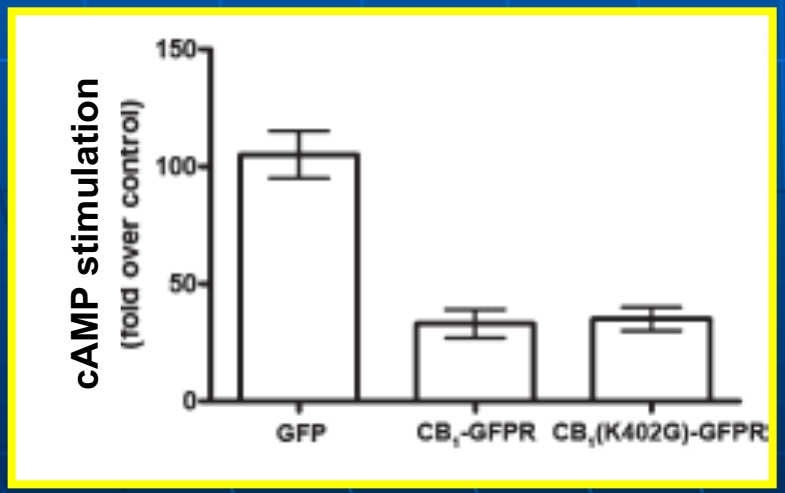


CB2R

Oddi & Dainese et al., 2011

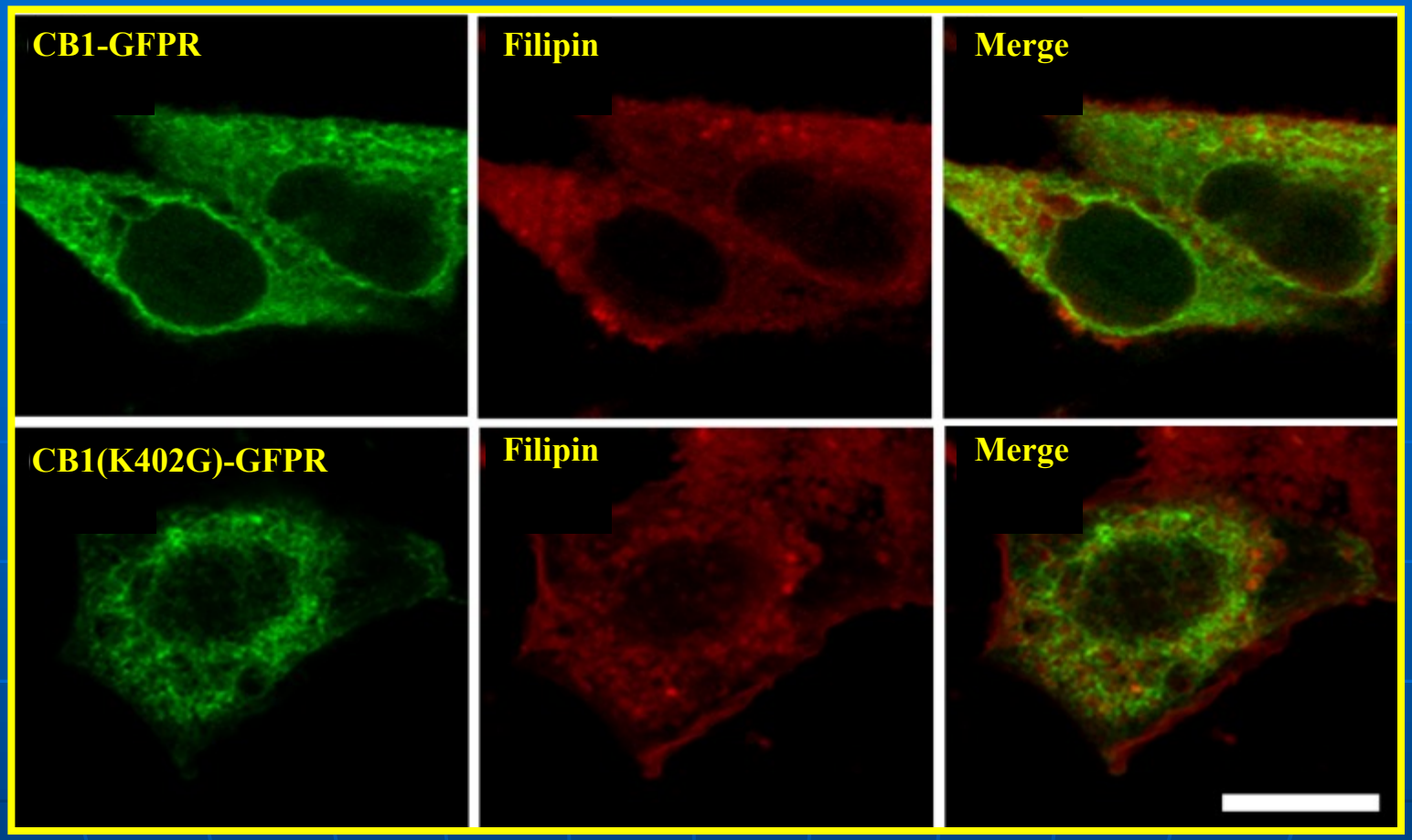
Functionality and intracellular distribution of WT and K402G CB1-GFPR





	WT	K402G
Ratio M/T	0.12 ± 0.02	0.09 ± 0.02
DRM remnant (%	20 ± 5	25 ± 5

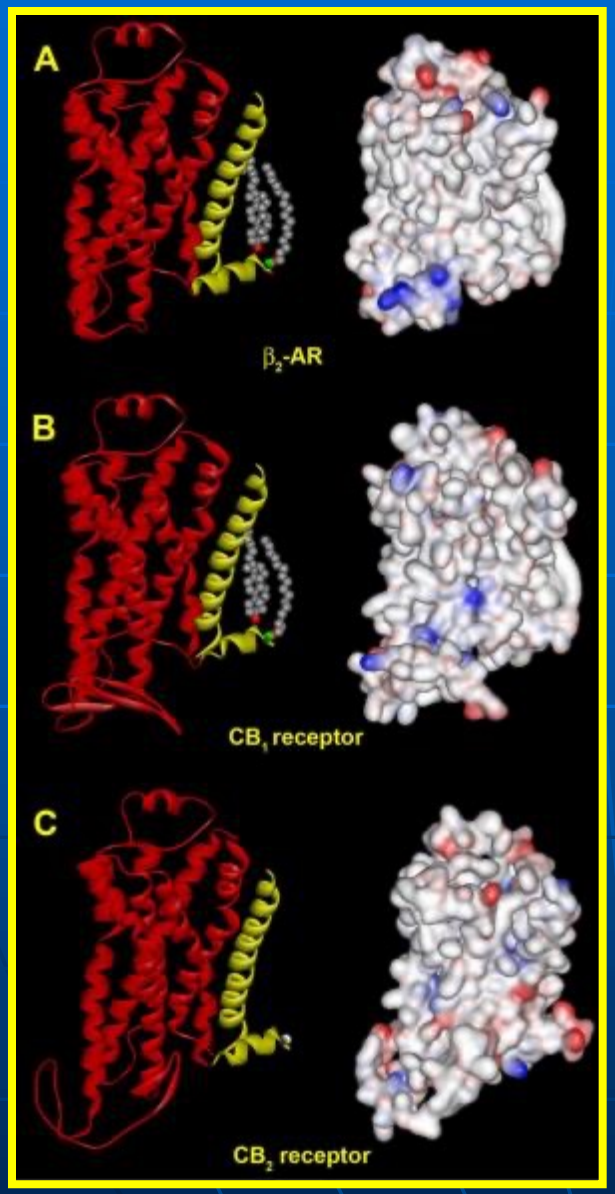
Quantitative colocalization of CB1-GFPR and filipin (cholesterol binder) on the plasma membrane

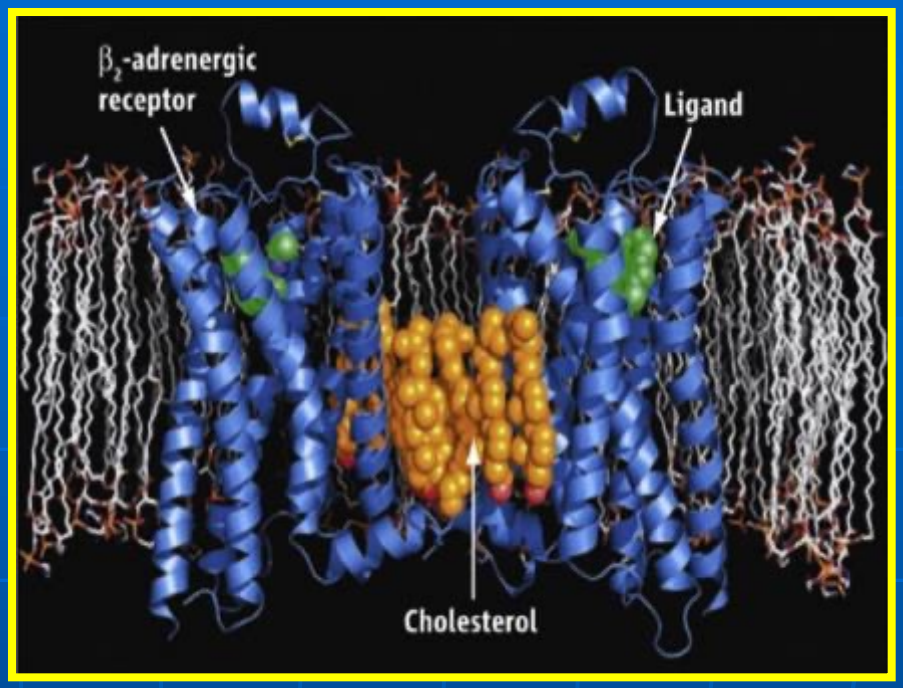


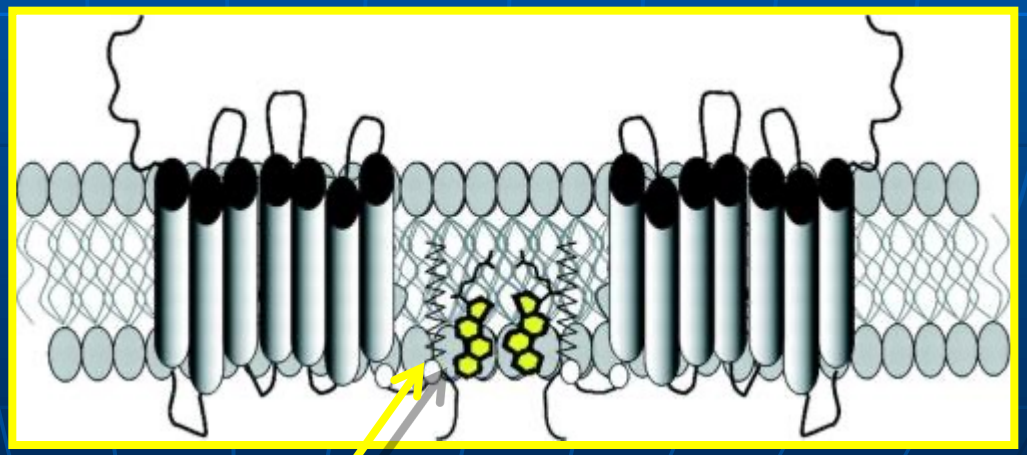
Receptor	M/T ratio	DRM remnant (%)	Pearson's correlation coefficient	Intensity correlation quotient
CB_1-GFPR	0.12 ± 0.02	20 ± 5	0.733 ± 0.039	0.390 ± 0.016
CB_1(K402G)-GFPR	0.09 ± 0.02	25 ± 5	0.560 ± 0.040*	0.274 ± 0.032*

*$p < 0.05$ vs. CB_1-GFPR.

β_2-AR and CB1 receptors share putative palmitoylation sites

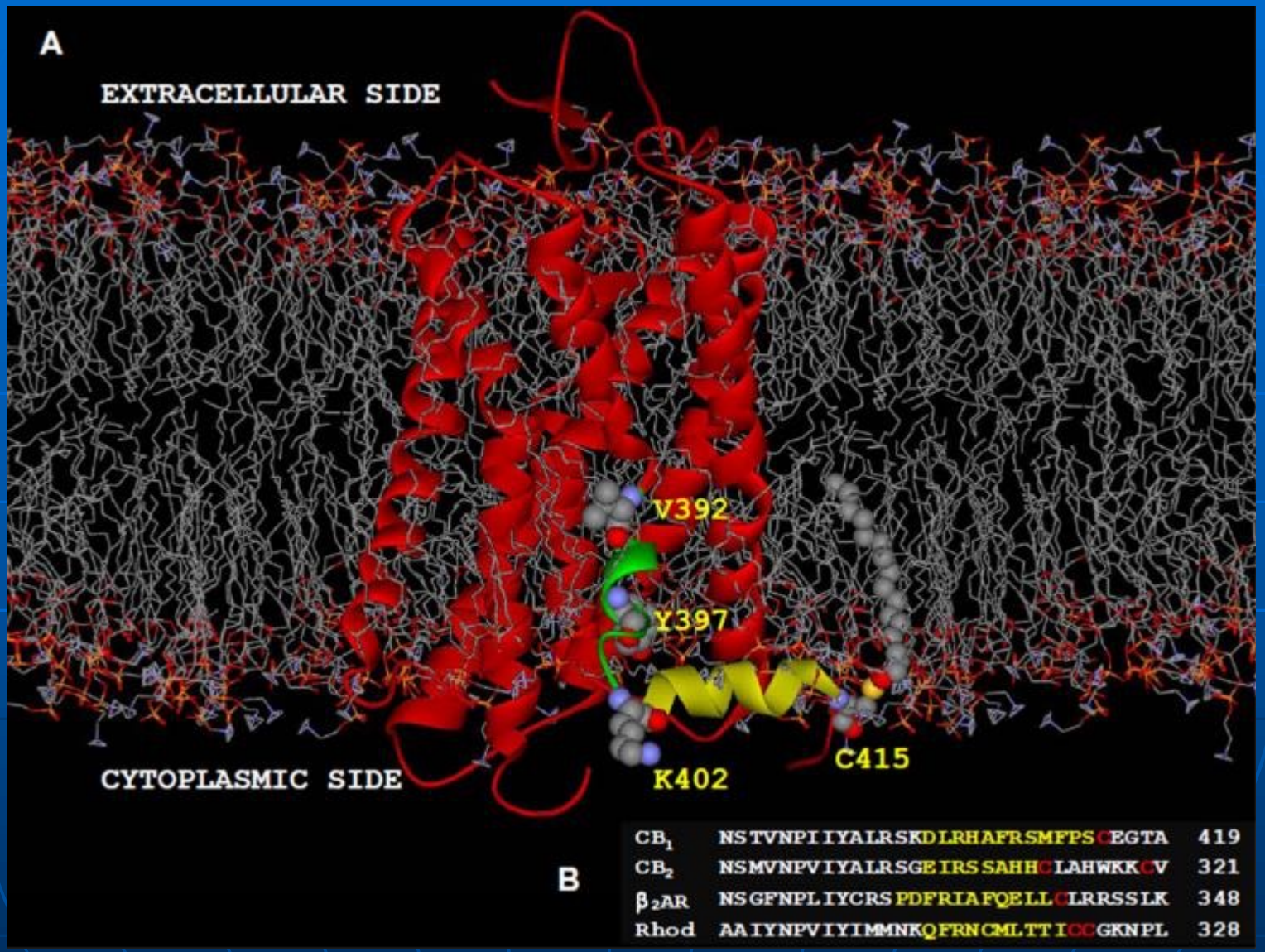




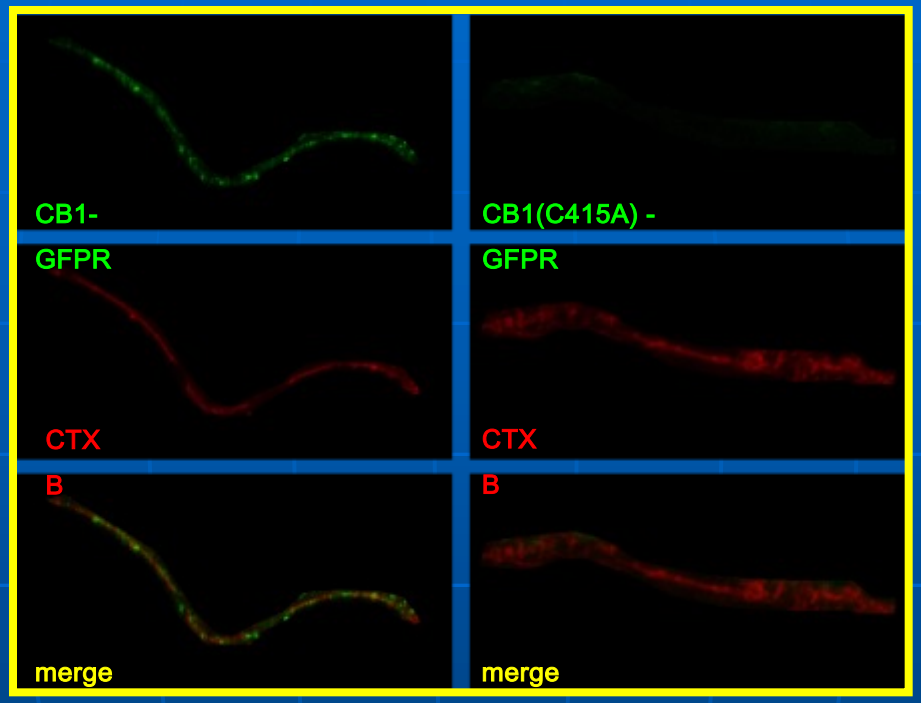


Palmitate residue

Putative palmitoylation site in CB1R



Cys415 palmitoylation is involved in targeting CB1 receptor to the plasma membrane

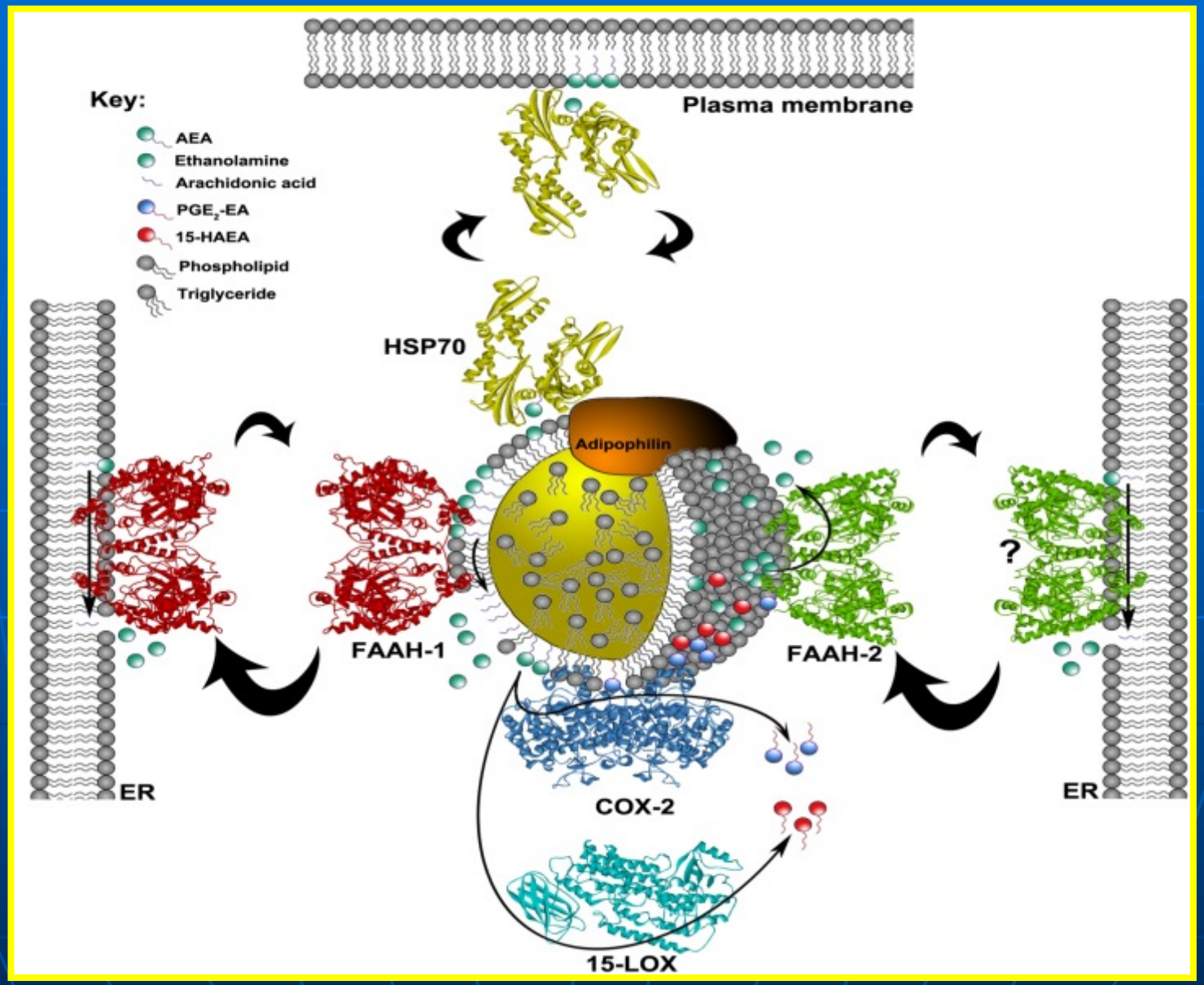


Protein	Pearson's Correlation Coefficie	Overlap Coefficient	Intensity Correlation Quotient
CB1-GFPR	0.74 ± 0.08	0.74 ± 0.07	0.214 ± 0.035
CB1(C415A)-GFPR	0.27 ± 0.07*	0.27 ± 0.04*	0.046 ± 0.009*

*p < 0.01 *versus* CB1R-GFP

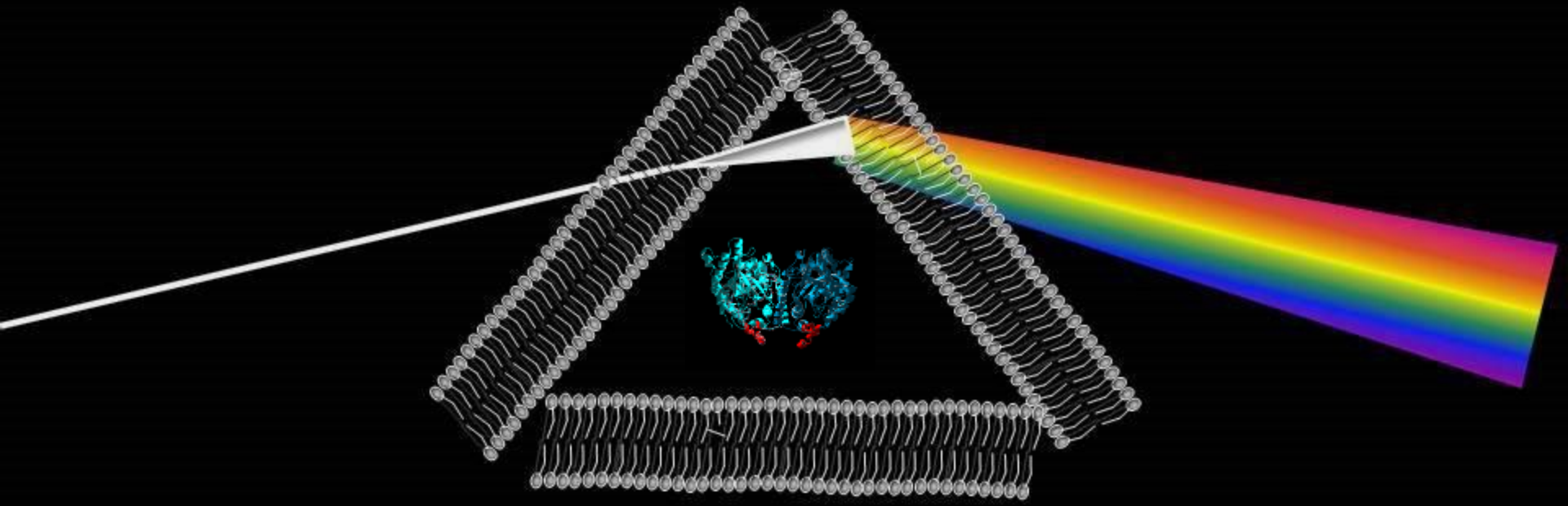
Oddi & Dainese et al., 2012

Lipid-lipid and lipid-protein interactions: trafficking of AEA as a control point of its signalling

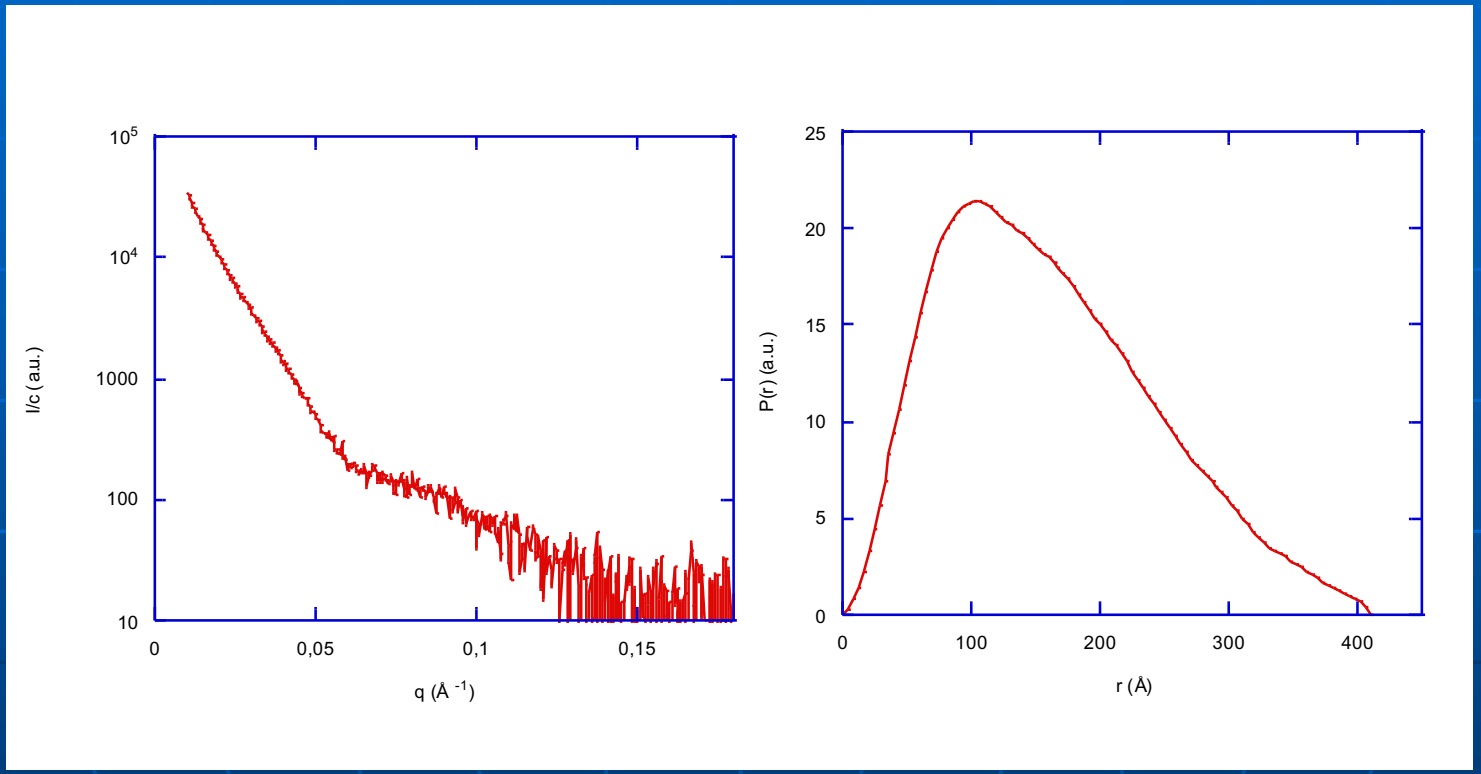


Maccarrone, Dainese & Oddi, 2011

STUDIES OF FAAH-LIPIDS INTERACTIONS BY COMBINING Fluorescence Resonance Energy Transfer (FRET), Small Angle X-ray Scattering (SAXS) and *in silico* APPROACHES



Determination of the oligomerization state of FAAH in solution by small angle X-ray scattering (SAXS)



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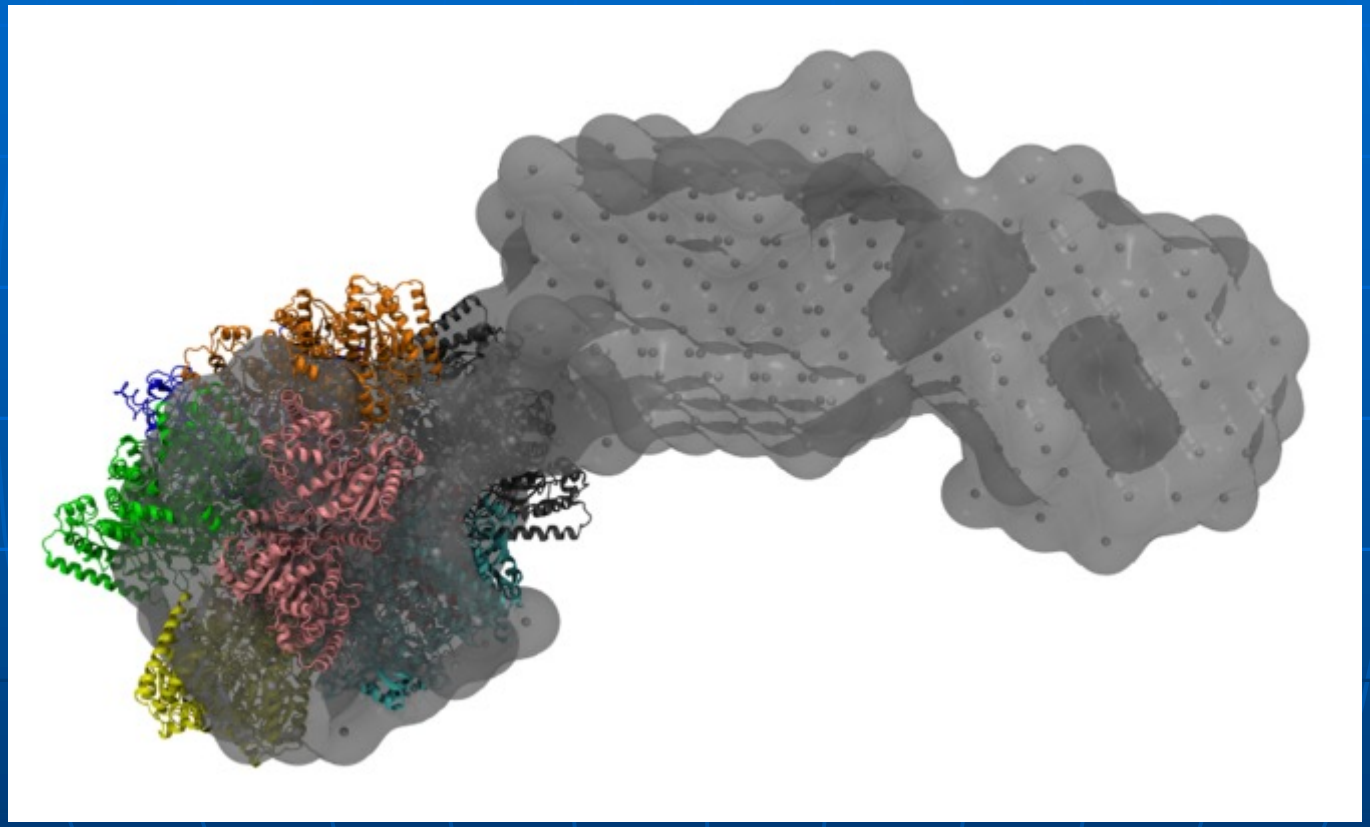
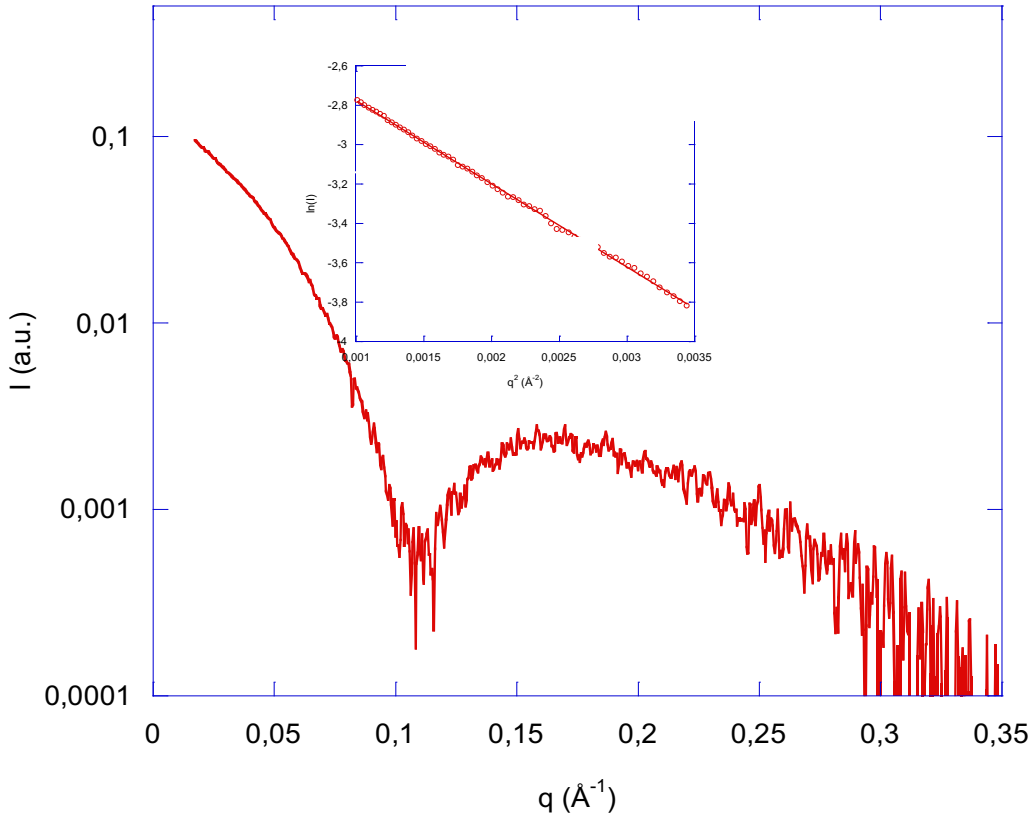
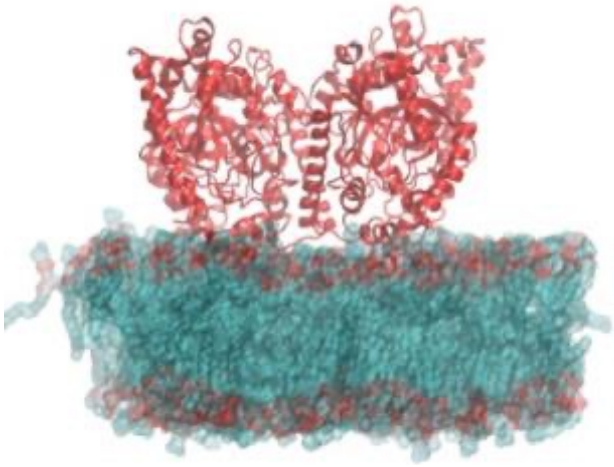
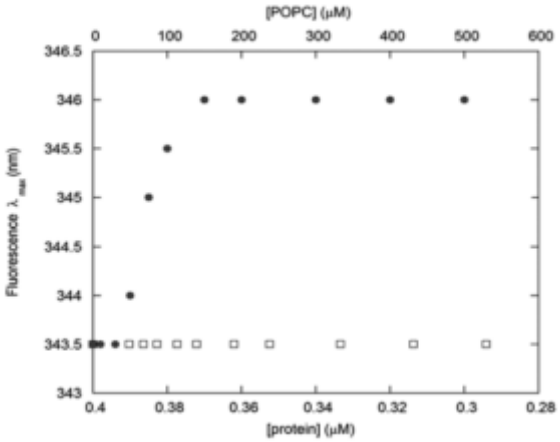
Determination of the oligomerization state of FAAH in solution by small angle X-ray scattering (SAXS)



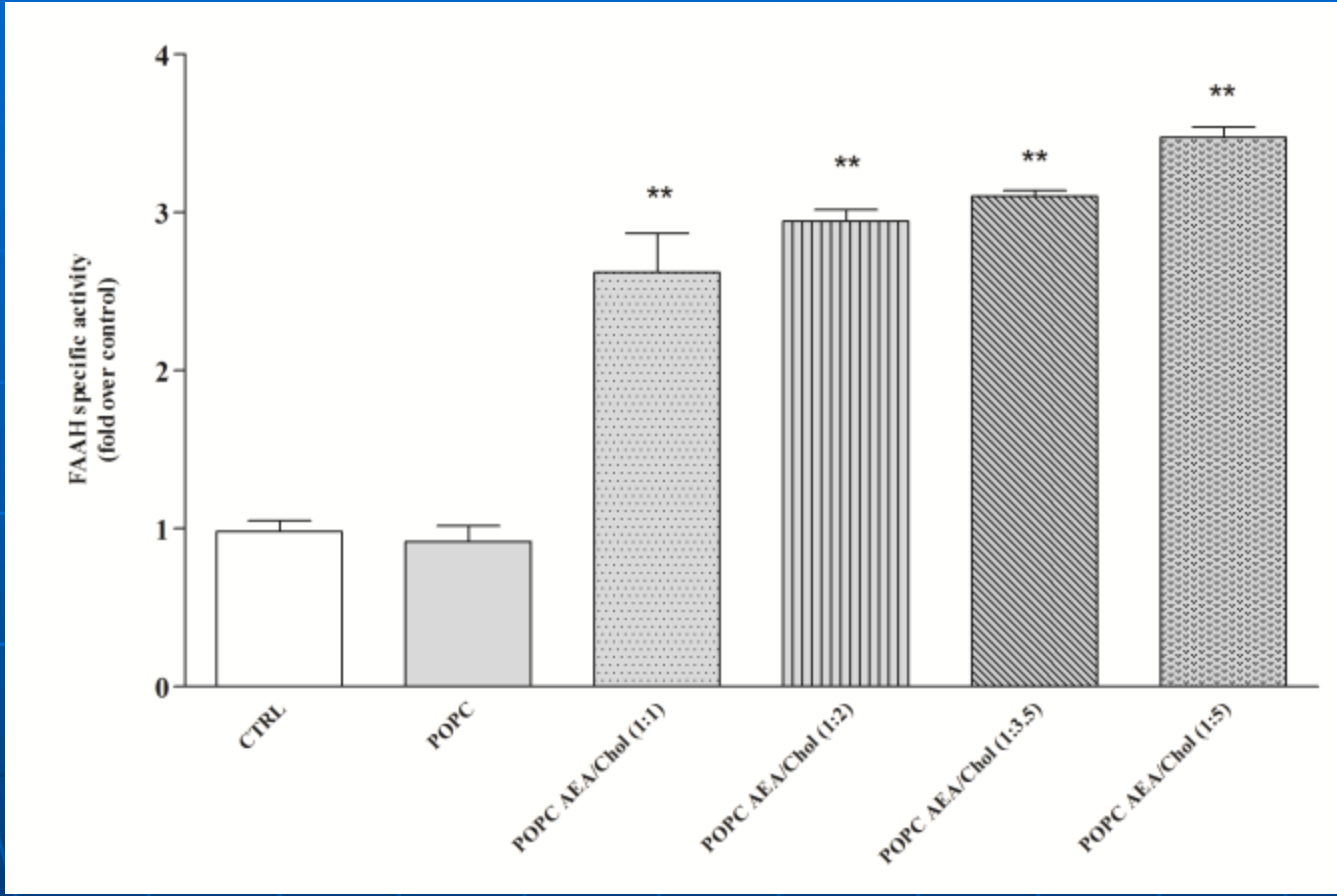
Membrane lipids dissociate these oligomers and stabilize FAAH dimer





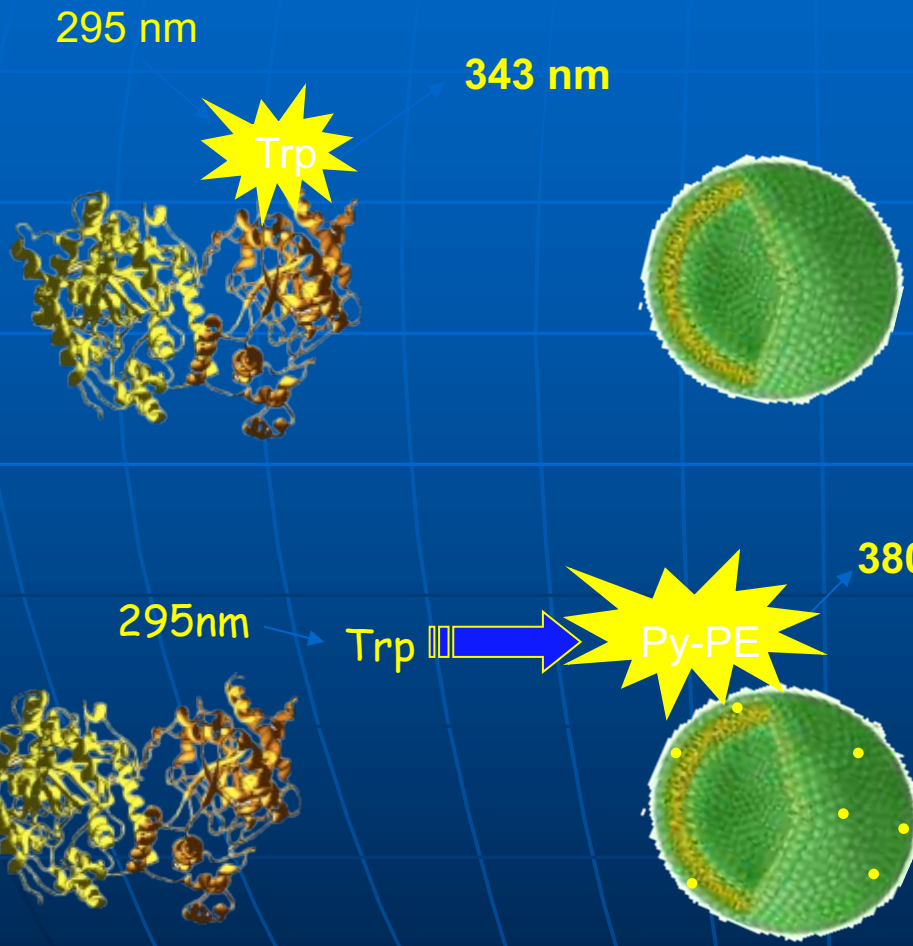


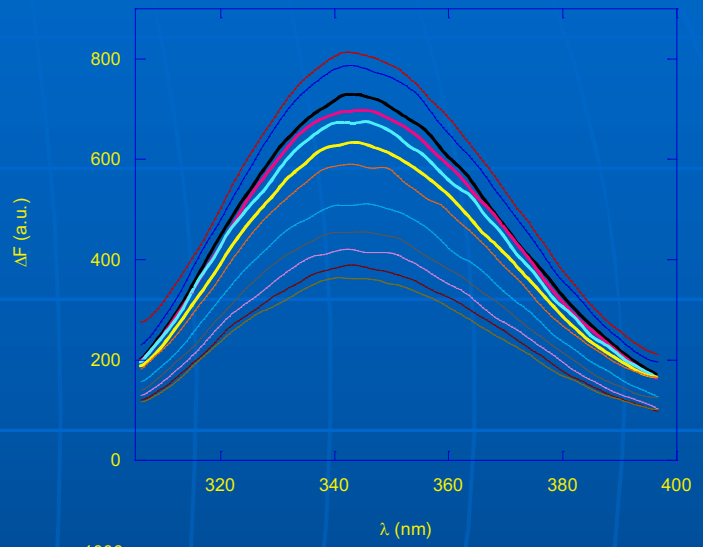
FAAH activity is strongly increased by membranes containing AEA and cholesterol

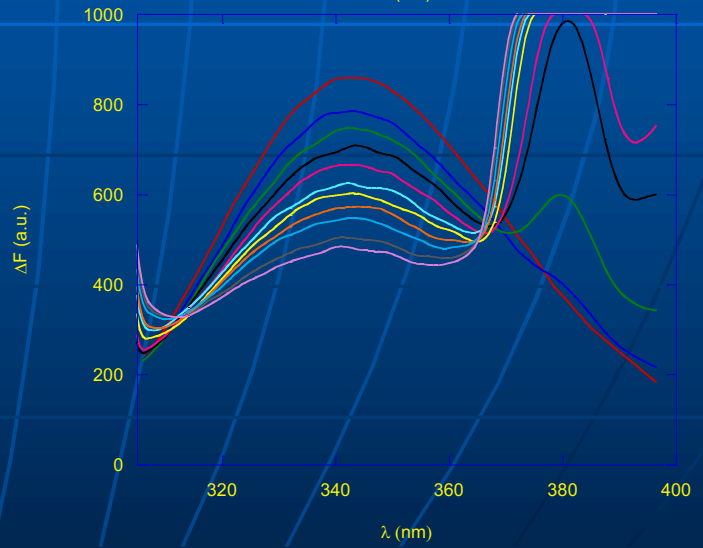


**p < 0.01 *versus* CTRL

Study of FAAH/membrane interaction by FRET

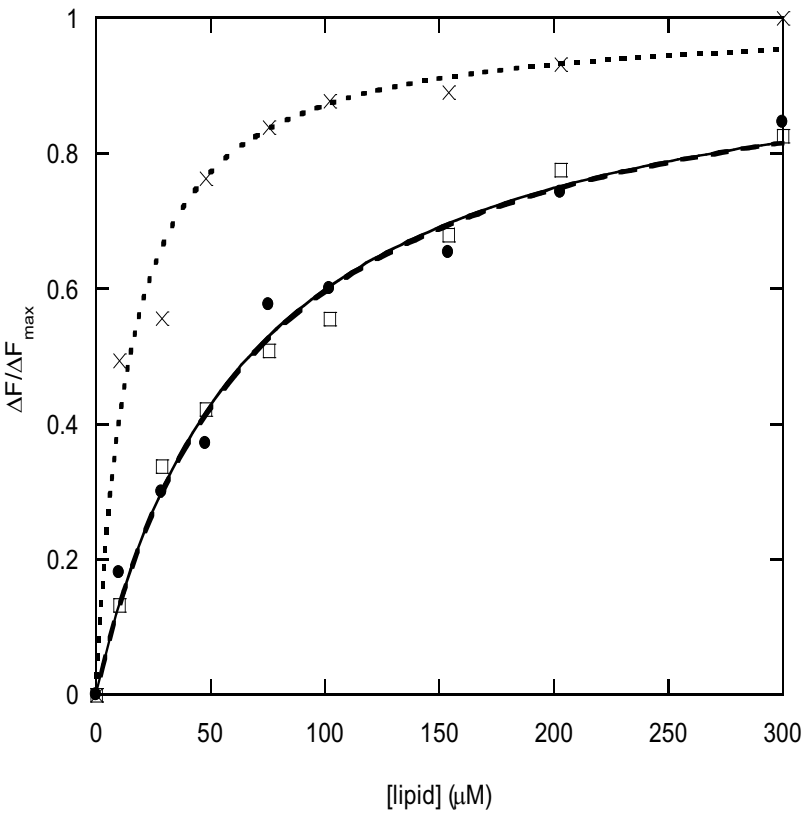


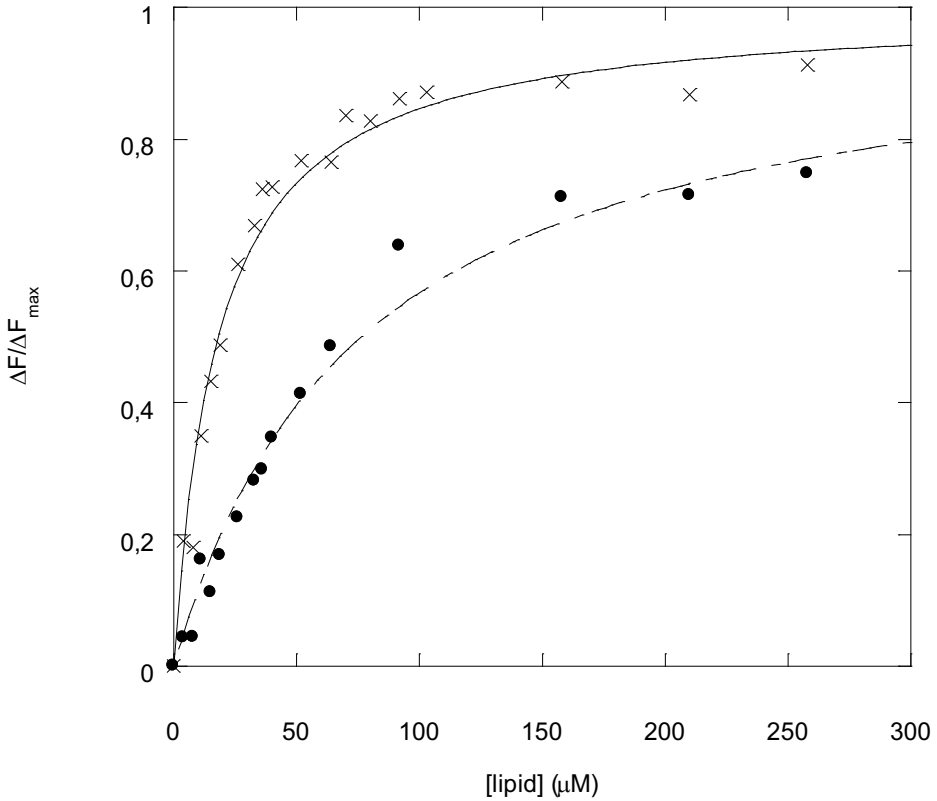




Angelucci CB, Sabatucci A, Dainese E. *Methods Mol Biol*. 2016;1412:267-76.

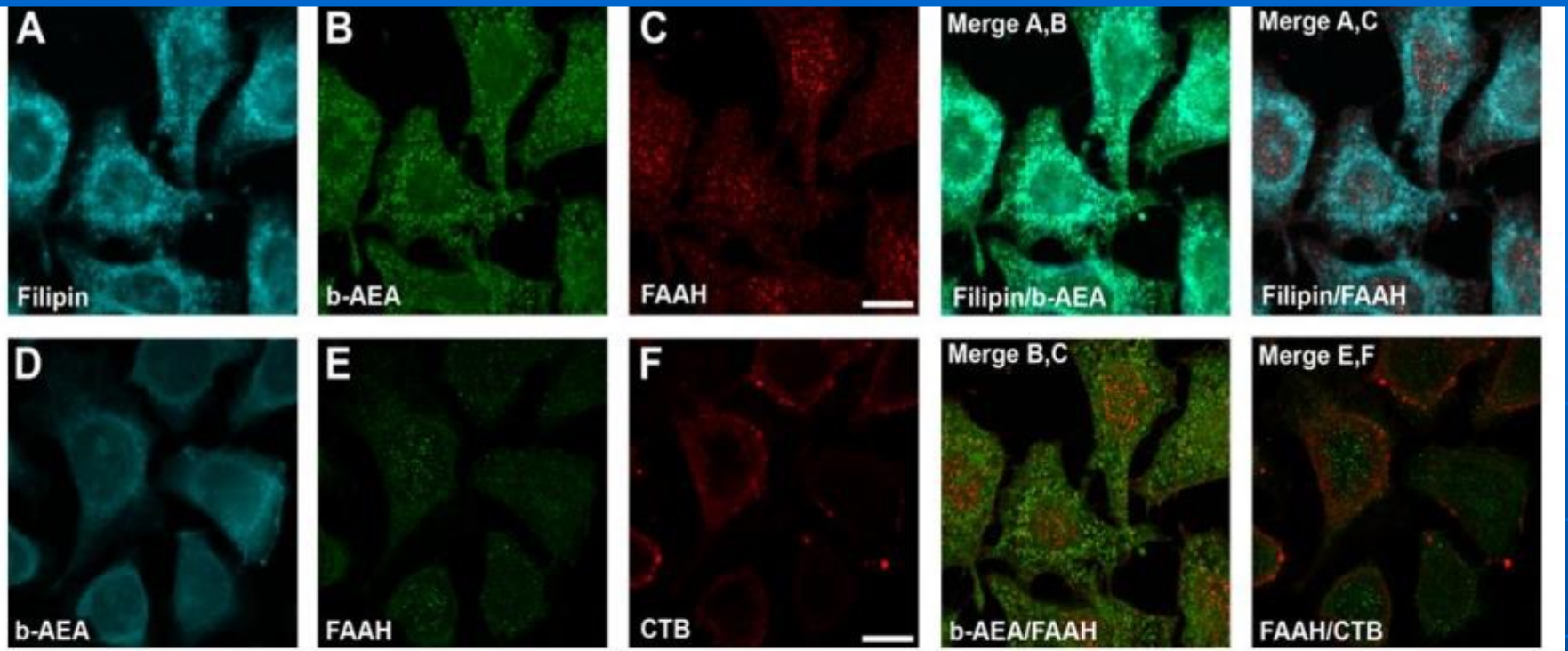
Higher membrane affinity of FAAH to ER membranes containing AEA and cholesterol





Dainese E et al., (2014) Membrane lipids are key modulators of the endocannabinoid-hydrolase FAAH. *Biochem J.* ***457*****(3)**:463-472.

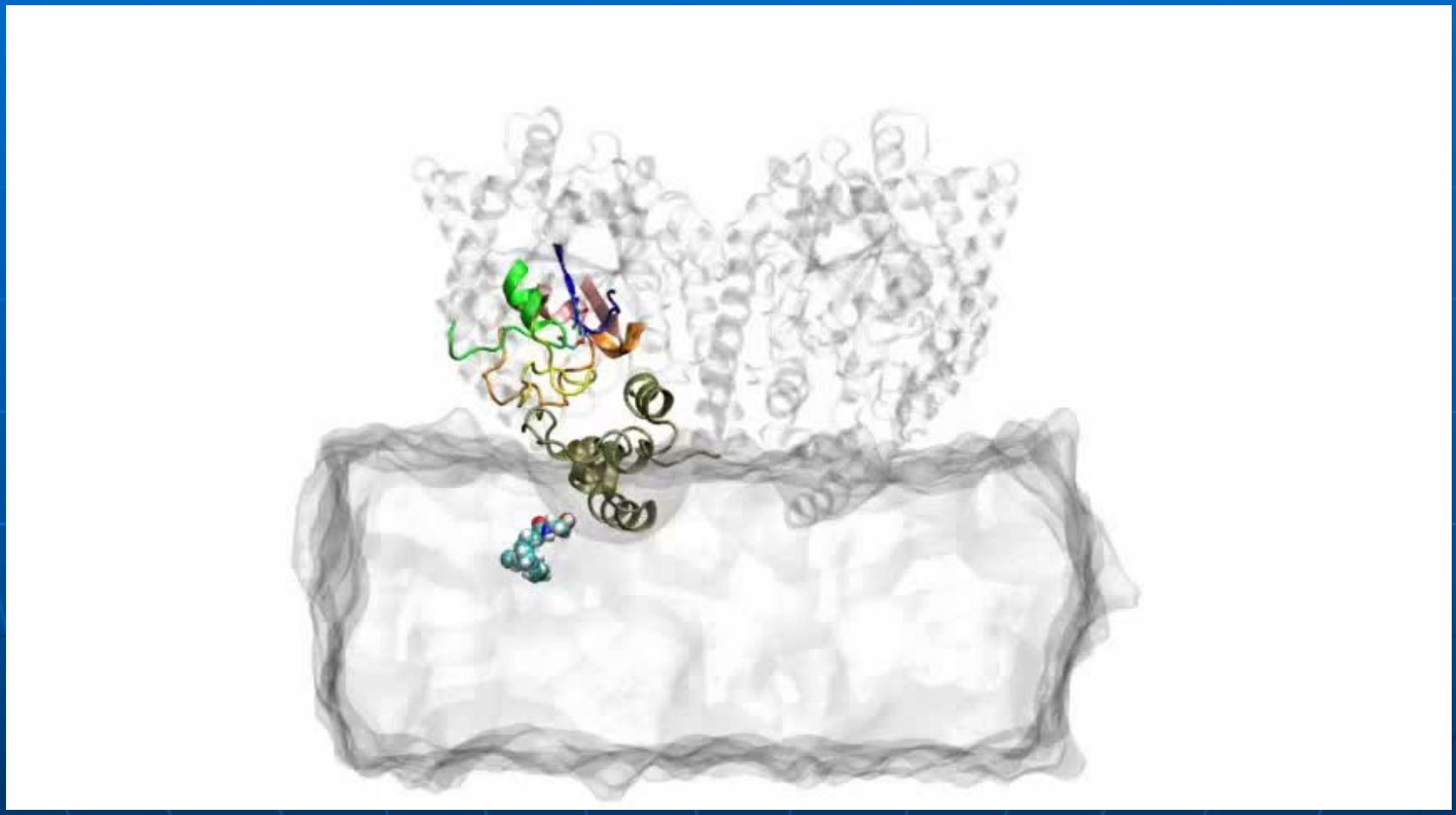
Confocal analysis of the cellular localization of FAAH



Parameter	Filipin/b-AEA	Filipin/rFAAH	b-AEA/rFAAH	FAAH/CTB
Pearson's correlation coefficient (R_r)	0.69 ± 0.03	0.43 ±0.02	0.51 ± 0.05	0.10 ± 0.02

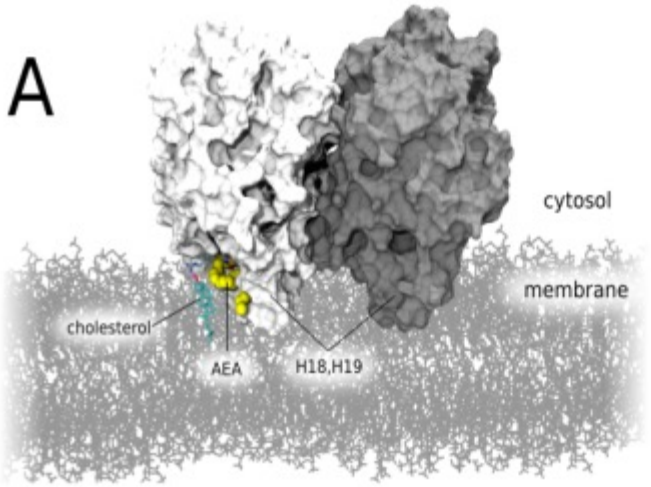
Dainese E et al., (2014) Membrane lipids are key modulators of the endocannabinoid-hydrolase FAAH. *Biochem J.* ***457*****(3)**:463-472.

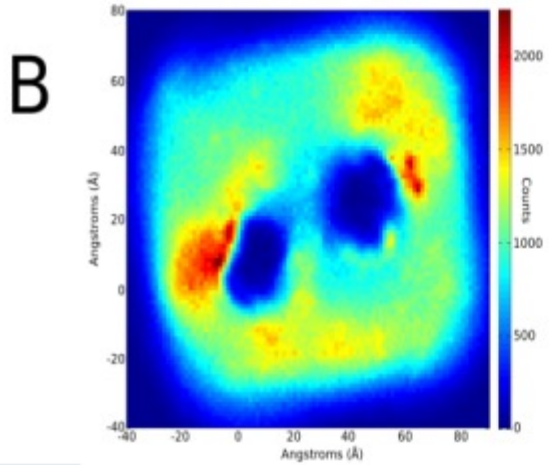
Molecular Dynamics (MD) of the full binding trajectory of AEA into the FAAH active site

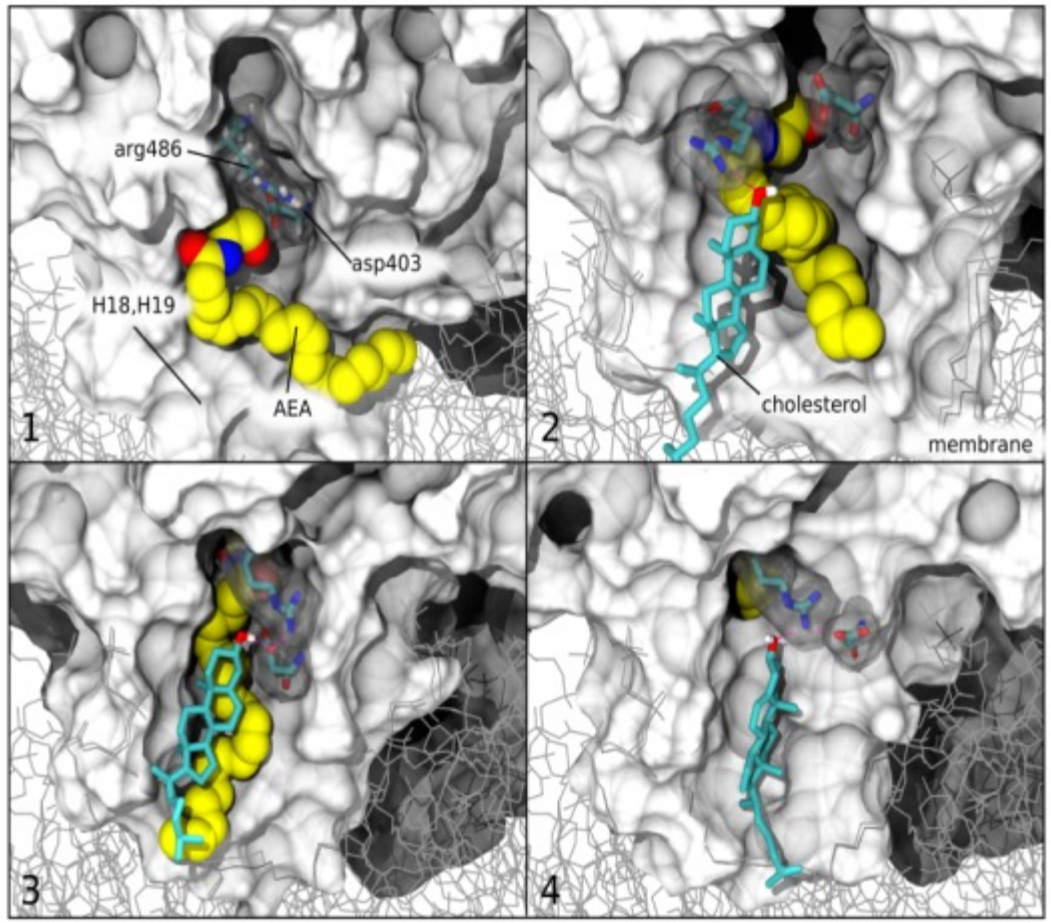


Dainese E et al., (2014) Membrane lipids are key modulators of the endocannabinoid-hydrolase FAAH. *Biochem J.* ***457*****(3)**:463-472.

MD simulations show that cholesterol facilitates the binding of AEA to FAAH by opening the membrane port

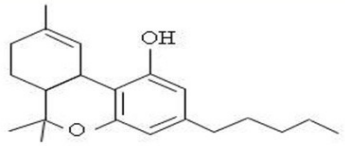
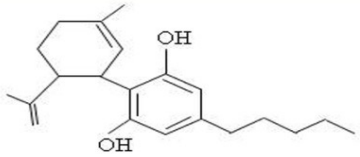
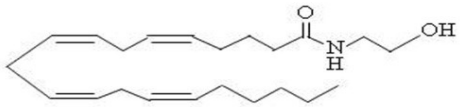
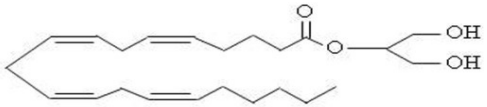
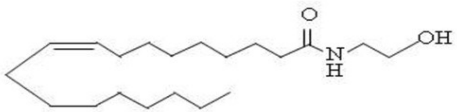
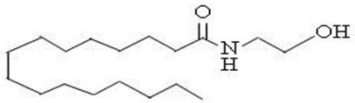
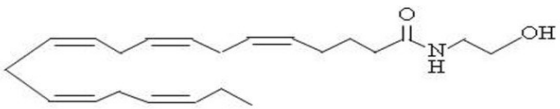
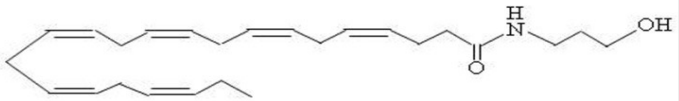






Dainese E et al., (2014) Membrane lipids are key modulators of the endocannabinoid-hydrolase FAAH. *Biochem J.* ***457*****(3)**:463-472.

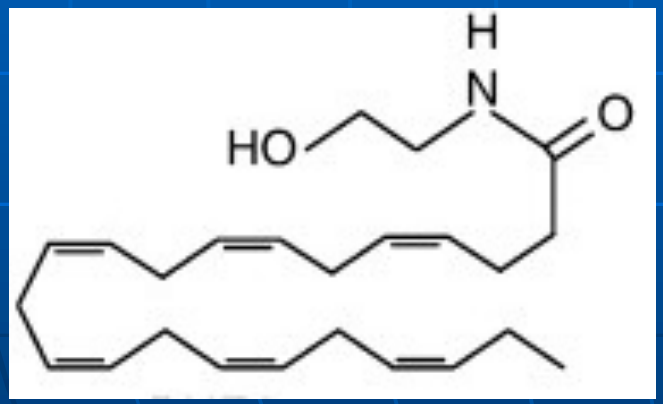
Phytocannabinoids and endocannabinoids (eCBs)

Phytocannabinoids	
 Δ⁹-tetrahydrocannabinol (Δ⁹-THC)	 Cannabidiol (CBD)
ω6 eCBs	
 *N*-arachidonoyletanolamine (Anandamide, AEA)	 2-arachidonoylglycerol (2-AG)
eCB-like compounds	
 *N*-oleoylethanolamine (OEA)	 *N*-palmitoylethanolamine (PEA)
ω3 eCBs	
 *N*-eicosapentaenoylethanolamine (EPEA)	 *N*-docosahexaenoylethanolamine (DHEA)

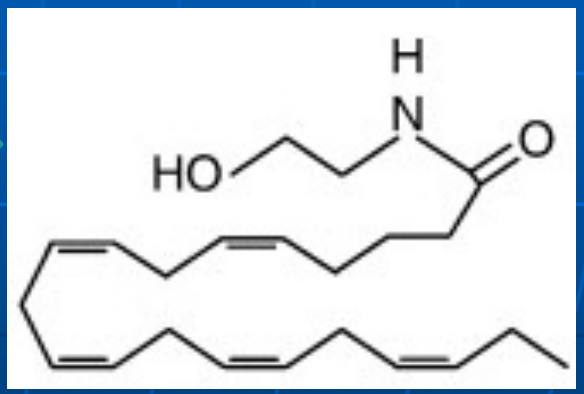
ω-3 ENDOCANNABINOIDS

- ANTI-CANCER AGENTS
- ANTI-INFLAMMATORY PROPERTIES

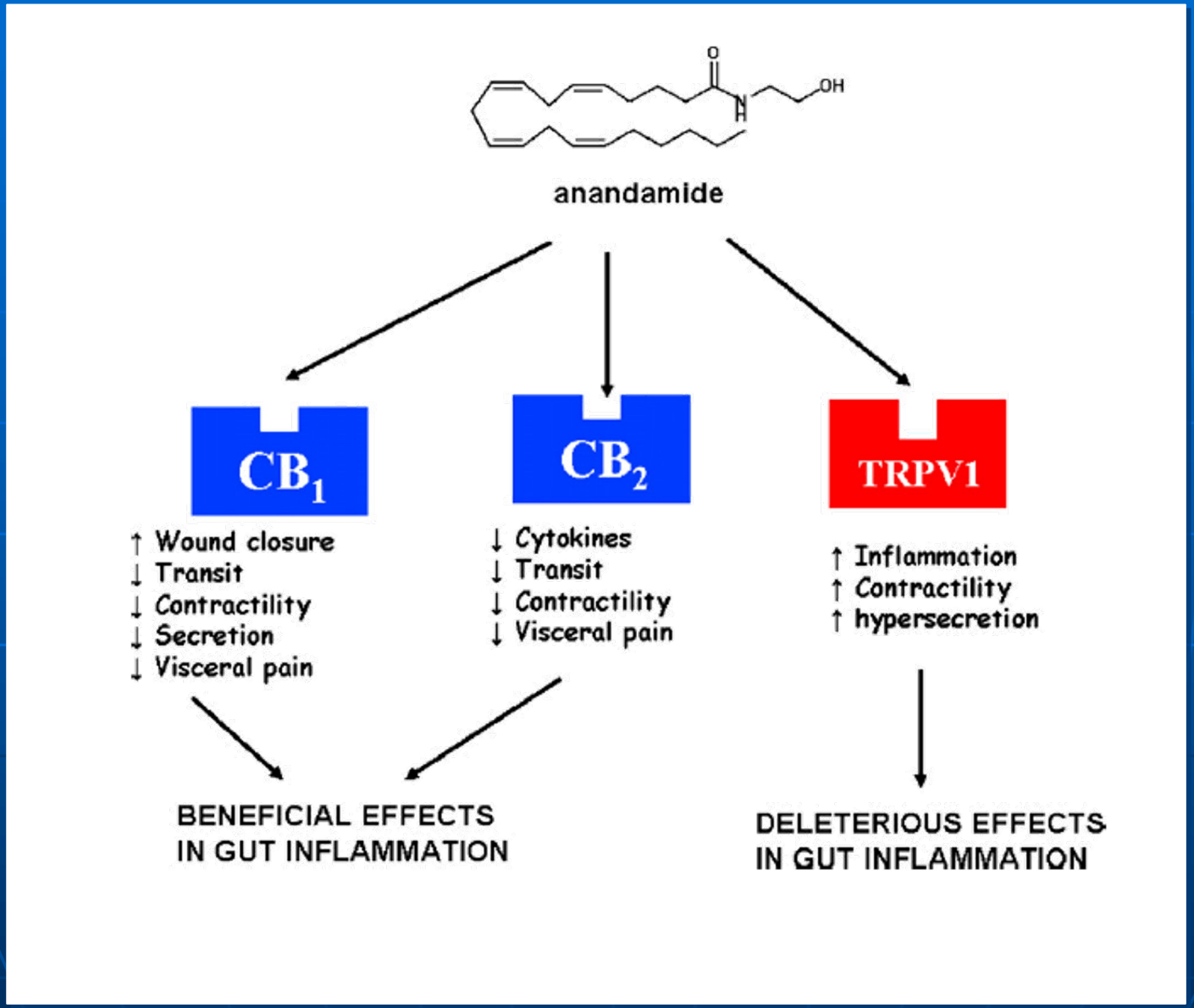
DHEA



EPEA



eCB system and the gastrointestinal tract (GI)



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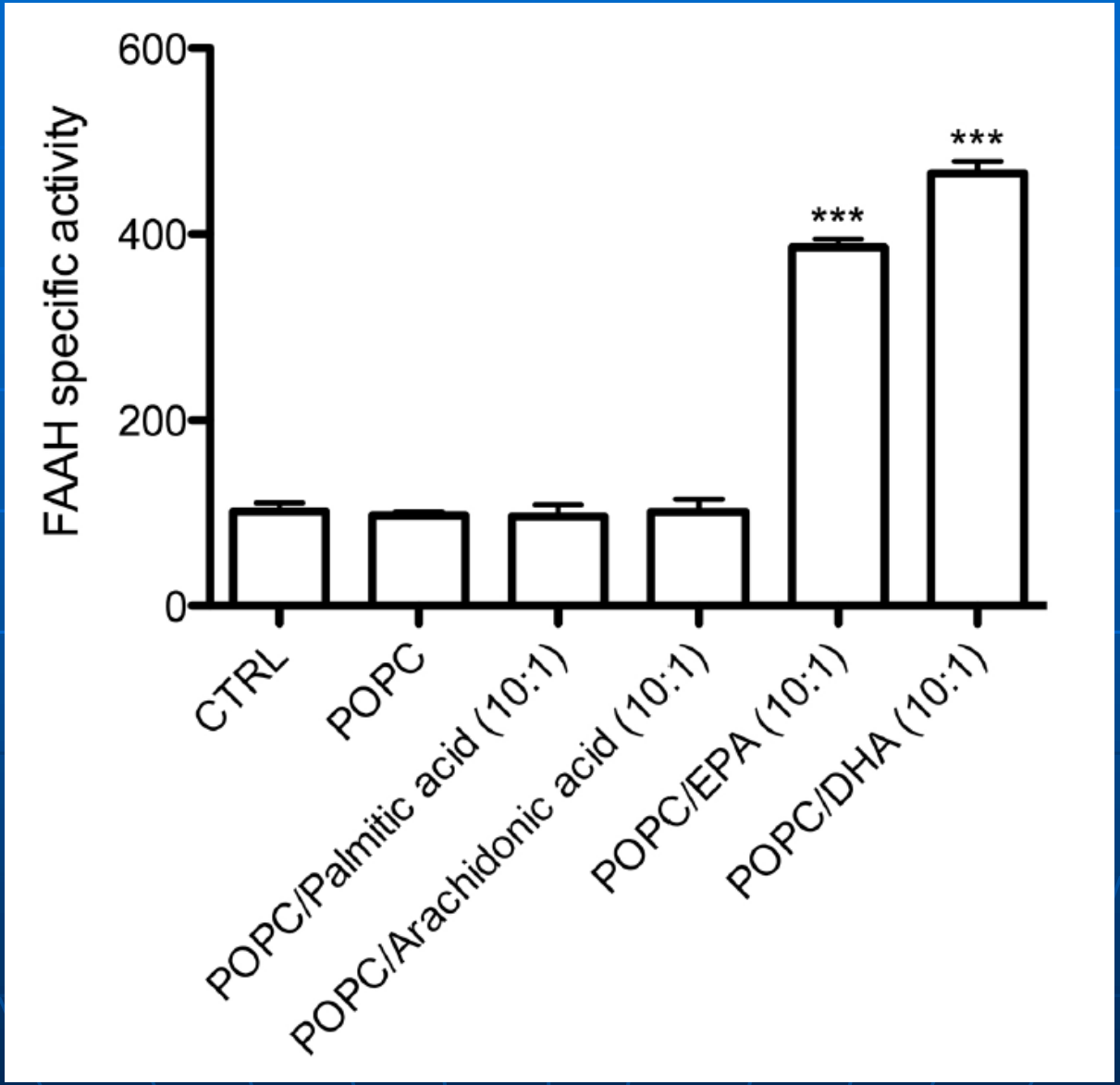
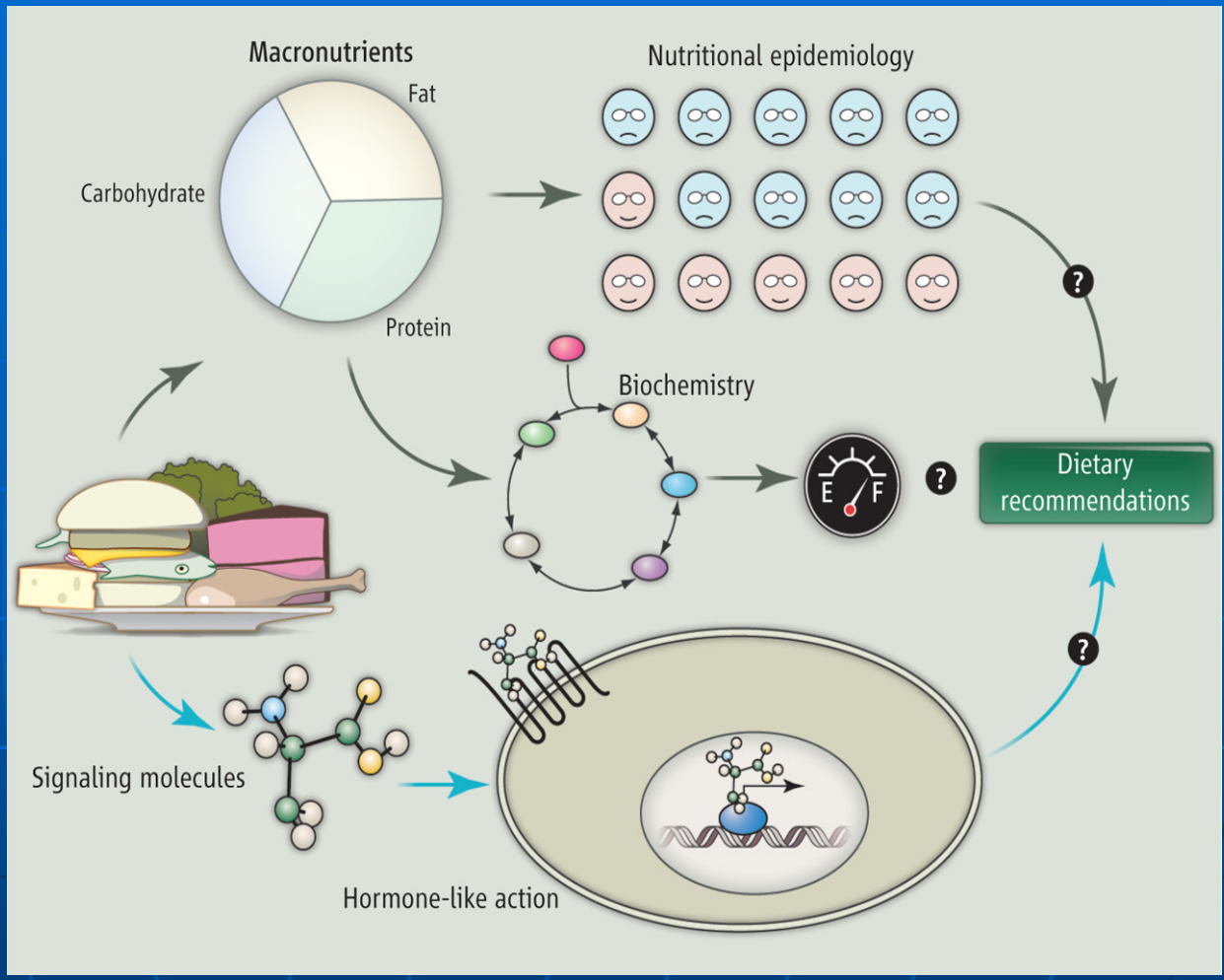
Role of endocannabinoids like molecules in Alzheimer disease



Food as a cocktail of hormones

tion, perhaps because considering food only in terms of its macronutrient content overlooks the complexities of how food interacts with our bodies.

A growing body of evidence suggests an alternative perspective. That is, circulating substrates derived from food have specific direct and indirect actions to activate receptors and signaling pathways, in addition to providing fuel and essential micronutrients. Ultimately food can be considered as a cocktail of "hormones." A hormone is a regulatory compound produced in one organ that is transported in blood to stimulate or inhibit specific cells in another part of the body. Hormones exert their effects on target tissues by acting on cell-surface receptors to alter activity through intracellular signaling cascades or via nuclear receptors to regulate gene transcription.



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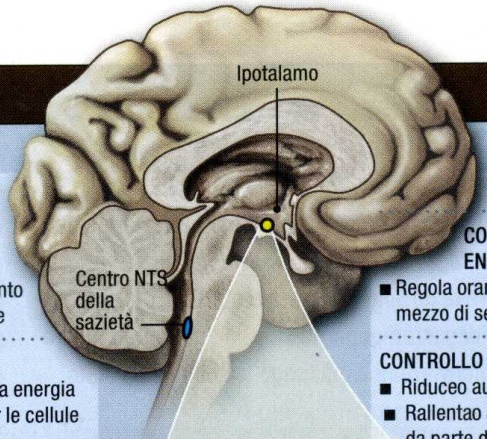
Centrale di controllo

Il cervello umano regola il peso corporeo integrando le informazioni sui bisogni energetici e lo stato delle riserve. Aree cerebrali specializzate stimolano l'appetito o la sazietà per far sì che più energia venga introdotta sotto forma di cibo, o per interrompere il processo quando si è mangiato a sufficienza. Col tempo, il cervello può anche gestire energia aumentandone o riducendone il consumo, o sottraendo energia ai processi fisiologici non essenziali per la sopravvivenza a breve termine.



INFORMAZIONI

ENERGIA IMMAGAZZINATA

- La leptina, un ormone prodotto dalle cellule adipose, indica quanto grasso contengono quelle cellule

STATO METABOLICO

- Il glucosio circolante rappresenta energia immediatamente disponibile per le cellule
- Vari indicatori dell'attività epatica segnalano che l'energia ingerita è in fase di elaborazione

STATO NUTRIZIONALE

- Segnali neurali e chimici provenienti dall'intestino indicano se gli organi digestivi sono pieni di cibo

RISPOSTE

CONTROLLO DELL'INTROITO ENERGETICO

- Regola orari e dimensione dei pasti per mezzo di segnali di appetito e sazietà

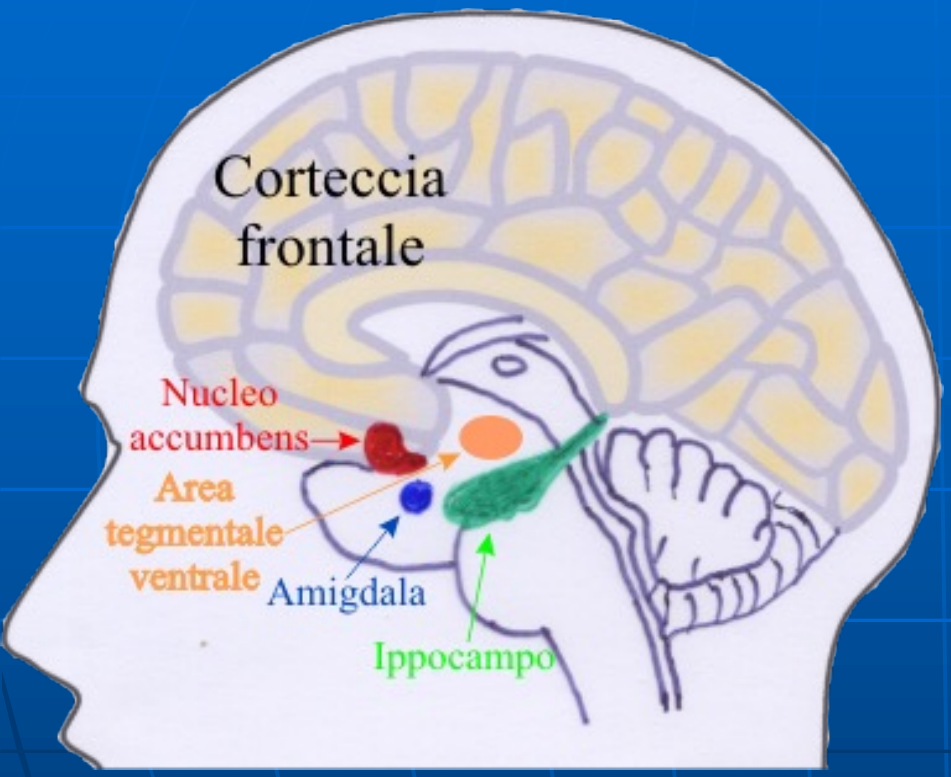
CONTROLLO DEL CONSUMO ENERGETICO

- Riduceo aumenta l'attività fisica
- Rallentao accelera l'impiego di energia da parte delle cellule
- Sopprime o ripristina crescita, riproduzione e funzione immunitaria

CONTROLLO DELL'APPETITO

Nel nucleo arcuato (ARC) dell'ipotalamo (*all'estrema destra*), indicatori del livello energetico e dello stato di nutrizione sotto forma di peptidi intestinali, come grelina e PYY, e ormoni quali leptina e insulina agiscono su gruppi di neuroni associati con appetito (*in marrone*) o sazietà (*in blu*). Ciascuna sostanza stimola (*frecce verdi*) o smorza (*frecce rosse*) le risposte neuronali. Quando sono stimolate, le cellule ARC rilasciano peptidi come NPY, AgRP e alfa-MSH, che agiscono su un secondo gruppo di neuroni ipotalamici i quali inducono appetito o sazietà. Leptina e insulina agiscono attraverso entrambi i tipi cellulari contemporaneamente per indurre sazietà, sopprimendo al contempo lo stimolo dell'appetito. Anche i segnali nervosi e il peptide intestinale colecistochinina (CCK) comunicano lo stato di nutrizione direttamente a un centro della sazietà (*a destra*) situato nel tronco cerebrale: il nucleo del tratto solitario (NTS).

Nutraceuticals and reward circuit



When this circuit is stimulated, active connections with other nuclei and areas of the base brain (amygdala, hippocampus). These regions process and retransmit to the nucleus accumbens different types of signals related to a pleasant and rewarding activities, such as food, sex, games but also the interpersonal and social relationships. The frontal cortex receives and integrates the information, coordinating the behavioral response.

eCBs control appetite, food intake and energy balance

The Endocannabinoid System and Its Relevance for Nutrition

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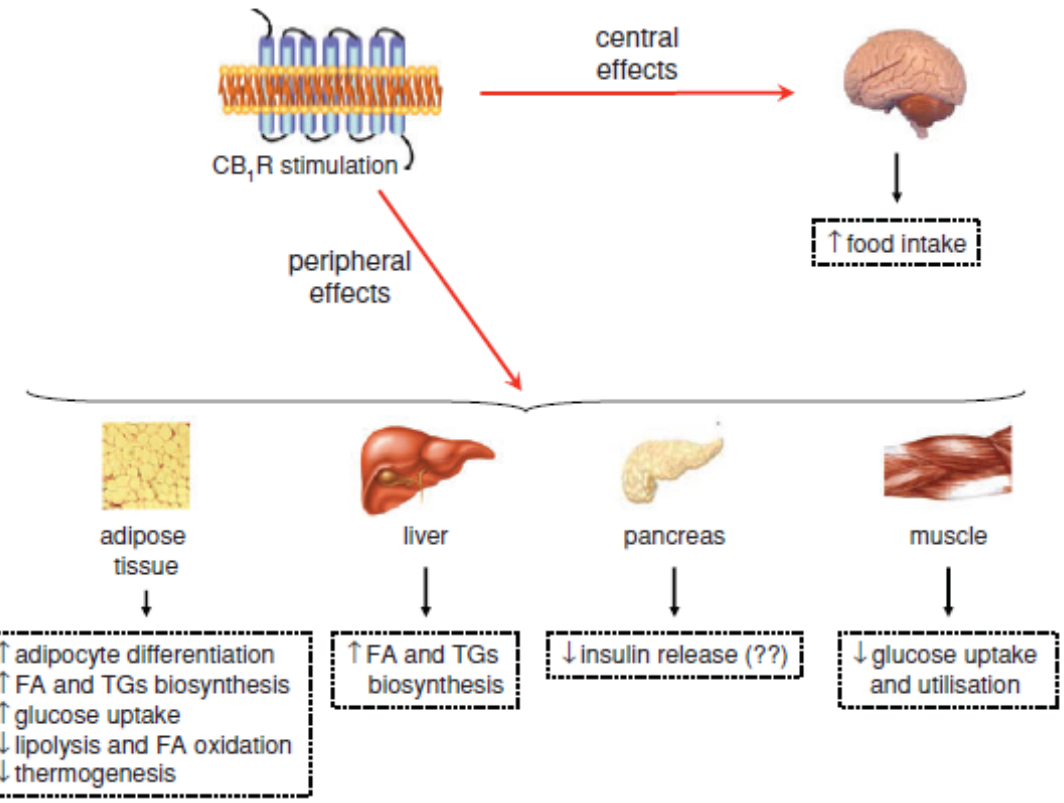

Figure 3

Central and peripheral effects of CB_1R activation on food intake and energy metabolism. FA, fatty acid; TGs, triglycerides.

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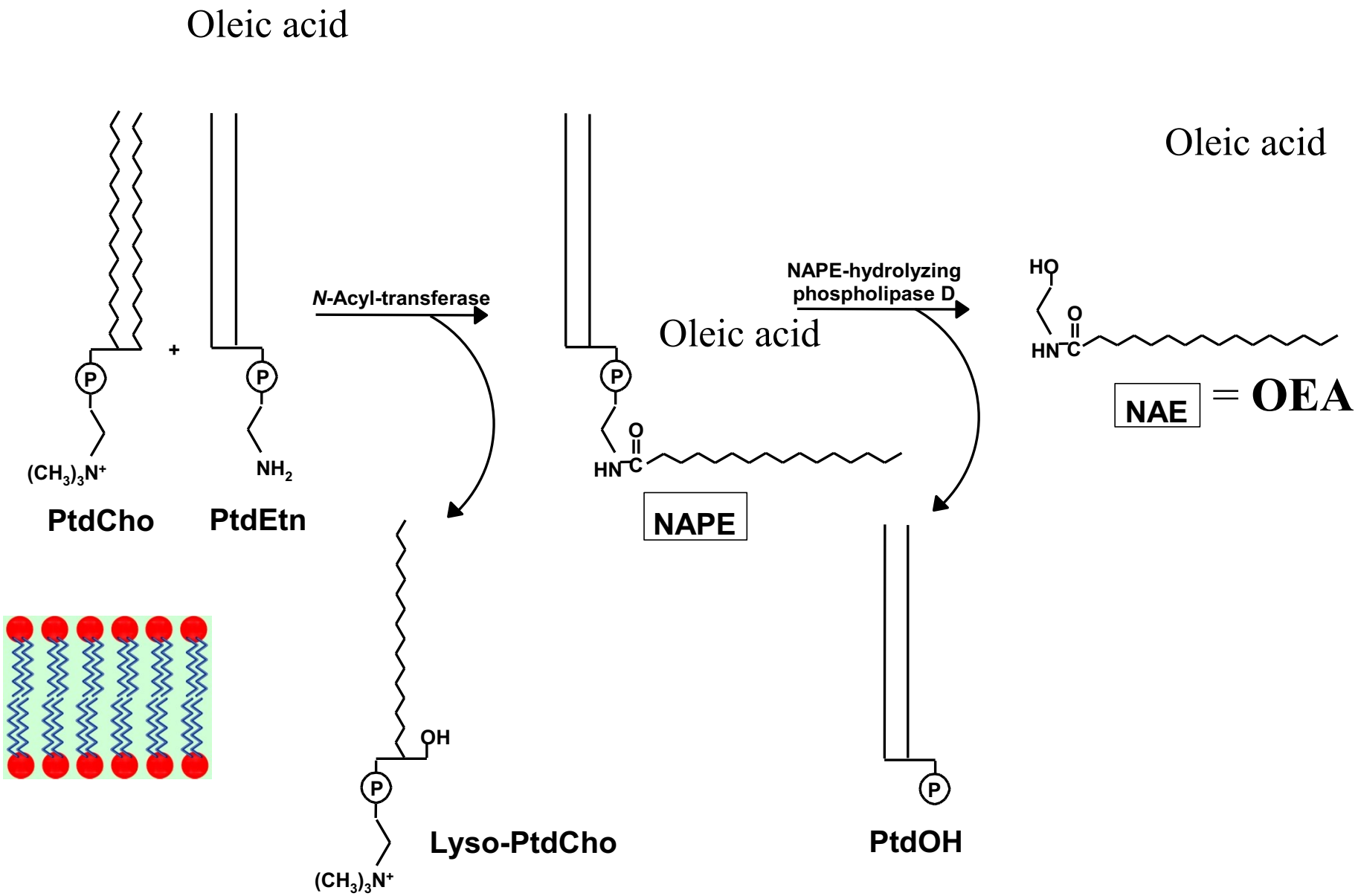
N-Oleoylethanolamine (OEA) is an anorexic lipid mediator regulated by feeding

Biosynthesis of OEA



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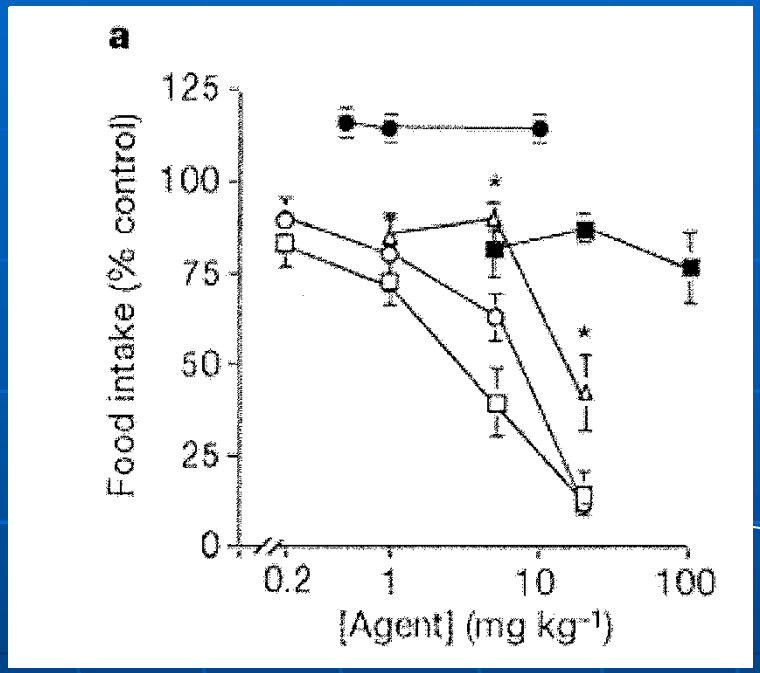
F.Rodríguez de Fonseca *et al.* (2001) *An anorexic lipid mediator regulated by feeding*. Nature 414;209



i.p. injection of:

Anandamide (20:4)

Oleic acid

N-Palmitoylethanolamine (16:0)

N-Elaidoylethanolamine (t-18:1)

N-Oleoylethanolamine (18:1) = OEA

OEA formed in the intestine may act locally via PPARα-activation, thus stimulating vagal sensory fibers that lead from the intestine to the brain appetite center.

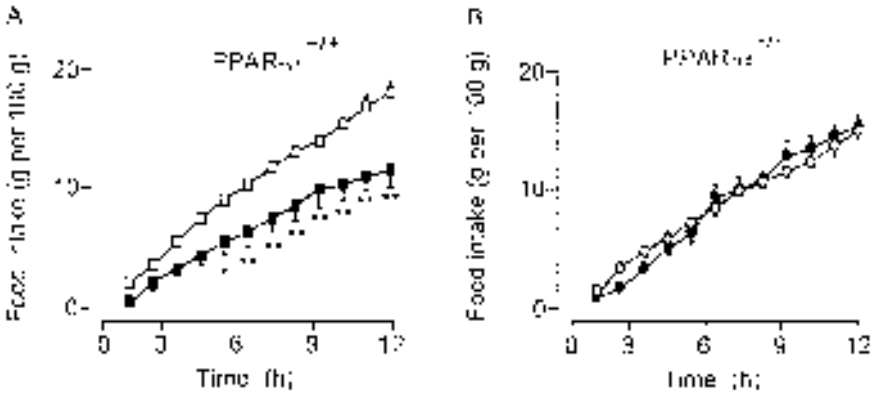
S.Gaetani *et al* (2003) *Modulation of meal pattern in the rat by anorexic lipid mediator oleoyl-ethanolamide.* Neuropsychopharmacology 28;1311

”OEA increases feeding latency and decreases meal frequency in rats”

Endogenous oleoylethanolamide may help maintain satiety in the post-ingestive state via activation of local PPARα. The signal is then mediated via vagal sensory fibers to the brain appetite center.

J.Fu *et al* (2003) *Oleoylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPARα.* Nature 425; 90.

Compound	EC50 (μM)
OEA	0.120
GW7647	0.006
Oleic acid	10
Fenofibrate	30
8(S)-HETE	0.2



Oral OEA decreases food intake in fasted rats

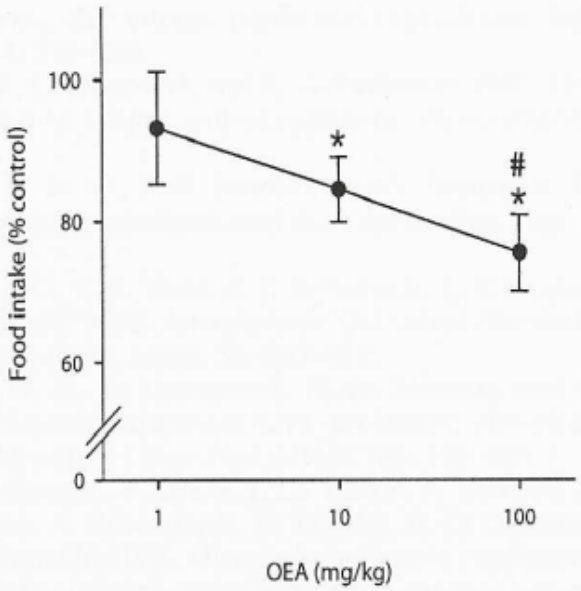


Fig. 1. Oleoylethanolamide (OEA) decreases food intake dose dependently upon oral administration. Twenty-four hour-starved rats were administered varying doses of OEA at 30 min before food presentation, and food intake was recorded at 90 min thereafter. Values (means ± SEM) are presented as percentages of control (100% = 6.4 ± 0.9 g, n = 12)

M.J.Nielsen *et al* (2004) J.Lipid Res. 45;1027

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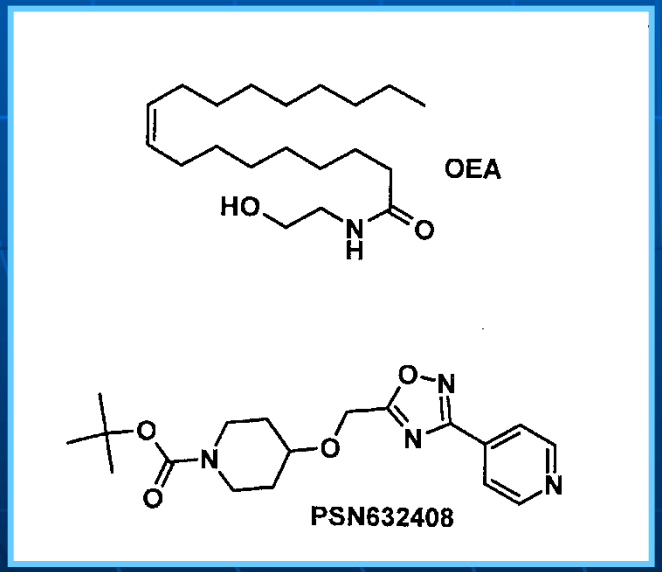
H.A. Overton *et al* (2006) *Deorphanization of a G protein-coupled receptor for OEA and its use in the discovery of small-molecule hypophagic agents.* Cell Metab. 3; 167-175



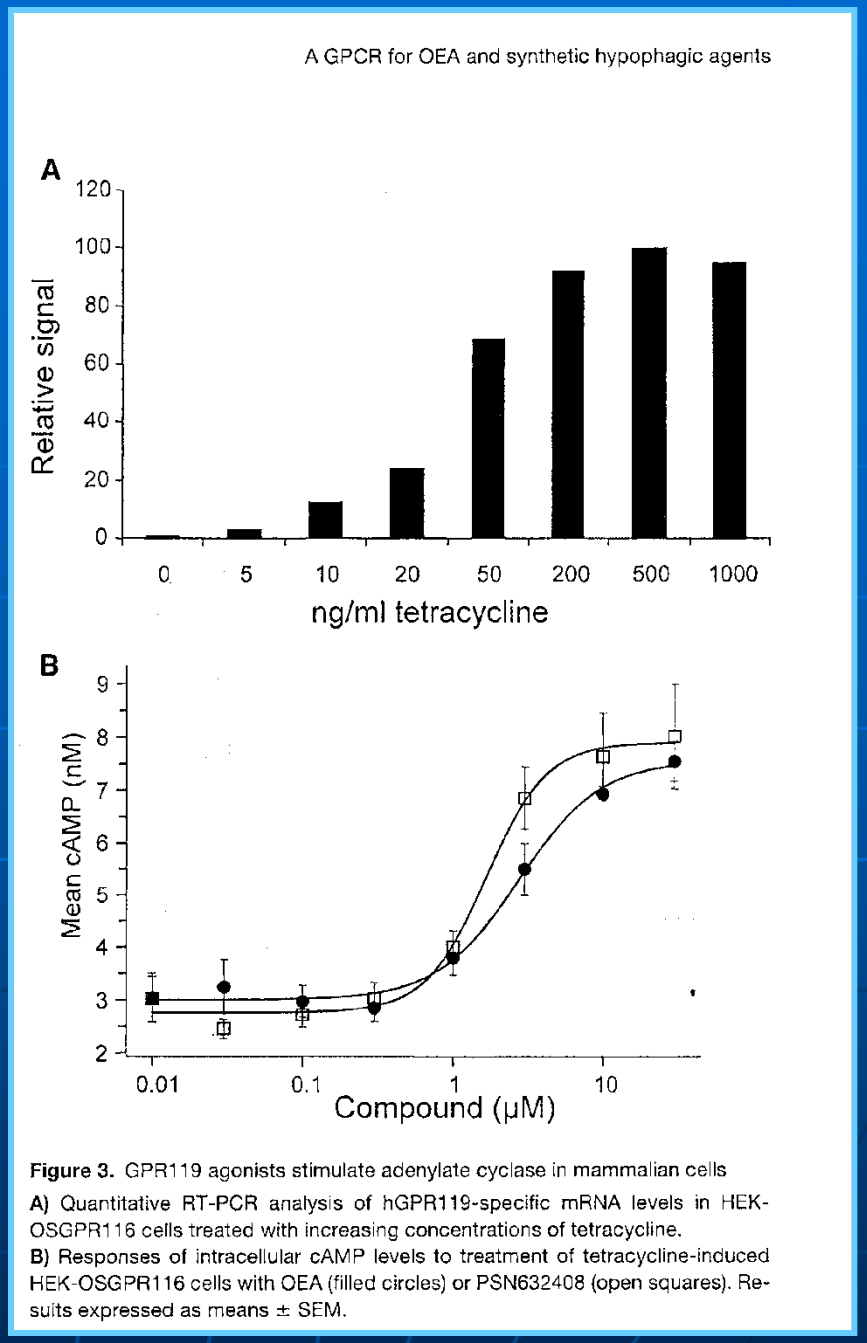


Figure 3. GPR119 agonists stimulate adenylate cyclase in mammalian cells

A) Quantitative RT-PCR analysis of hGPR119-specific mRNA levels in HEK-OSGPR116 cells treated with increasing concentrations of tetracycline.

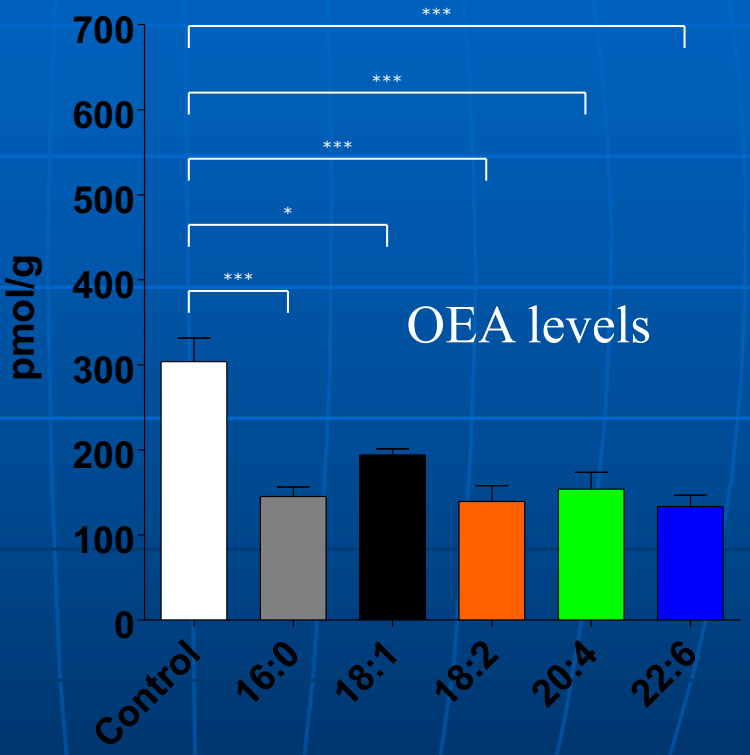
B) Responses of intracellular cAMP levels to treatment of tetracycline-induced HEK-OSGPR116 cells with OEA (filled circles) or PSN632408 (open squares). Results expressed as means ± SEM.

Dietary fat decreases OEA in Jejunum

All high-fat diets decreased levels of intestinal OEA in rats (A. Artmann et al, 2008)

Thus, high dietary fat intake may induce over-consumption of food through down-regulation of intestinal OEA levels

(Hansen & Diep, 2009)



Palm oil

Olive oil

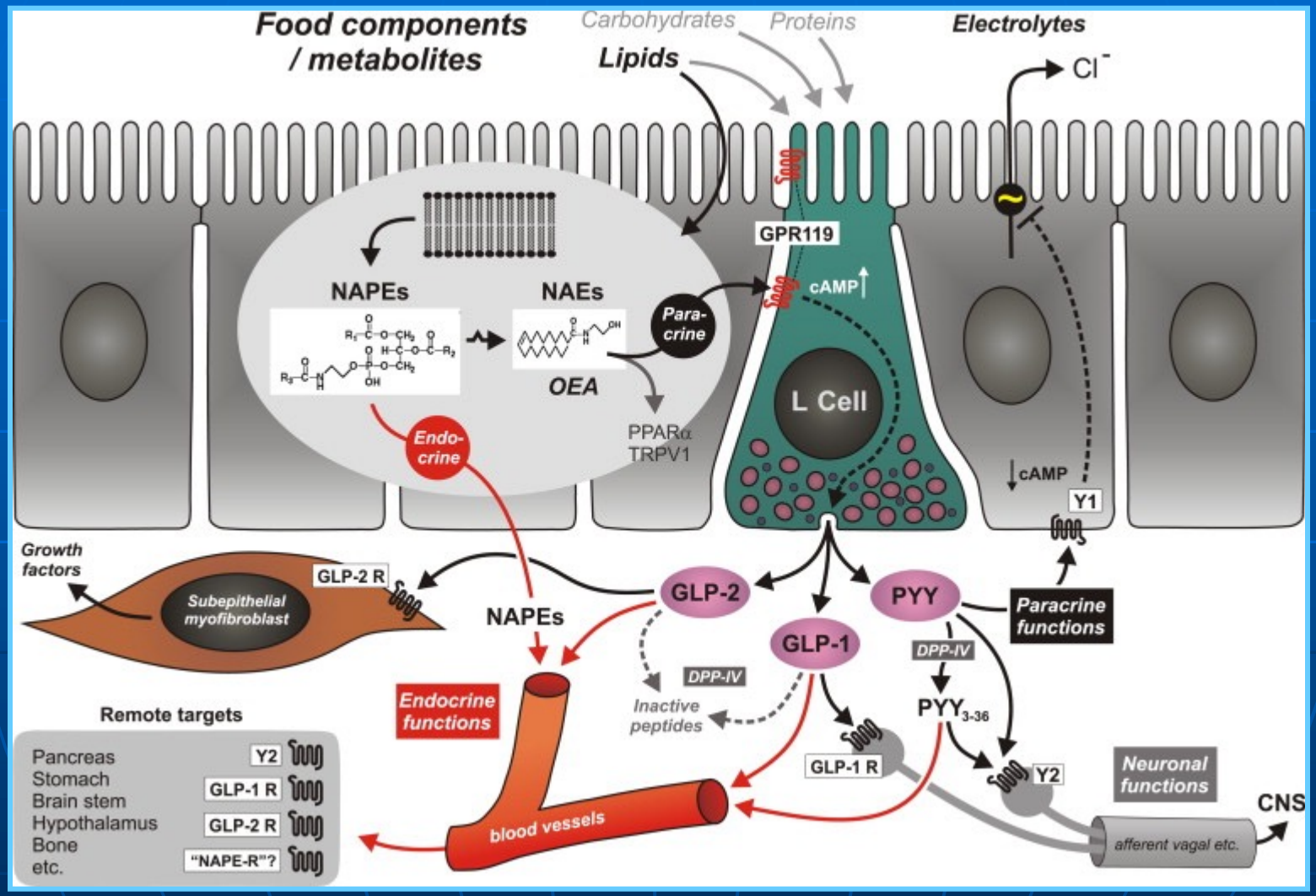
Safflower oil

Olive oil + 20:4

Olive oil + fish oil

Statistical Analysis: One Way ANOVA, followed by Tukey's Multiple Comparison Test (* P < 0.05; ** P < 0,01; *** P < 0.001).

OEA and the release of intestinal hormones (incretins)



Schwartz & Holst (2010) Cell Metab.

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CONCLUSIONS

Nutritional biochemistry is a science that involves the relationship of food and nutrients to health. The specific goal of this science is to improve human health by understanding the biochemical role of each nutrient in the diet.

The bioavailability of a nutrient depends on its concentration within the food but mainly on its chemical form affecting the intestinal absorption.

This is a fundamental rule governing the absorbtion of all nutrients in food:

-Carbohydrates, Proteins, Lipids;

-Vitamins and oligoelements;

-Antioxidant molecules and **NUTRACEUTICS**

Antioxidant activities

In vitro (i.e. within the food)

-Total antioxidant activity;
- Total polyphenols content (etc.)

Food stabilization effects:
-Reduced amount of reactive oxygen species in food;
- Reduced activities of pro-oxidant enzymes in food;
-Reduced amount of lipid peroxides in food;
- High quality of food.

Intestinal barrier

In vivo (e.g. within the body)

- Specific molecules derived from digestion (not always the same observed *in vitro*);
- Effects on anti- or pro-oxidant enzymes.
- Modulation of genes involved in lipid biosynthesis

Effects on health:
- Cell antioxidant activity;
- Reduced amount of oxidized LDL;
- Contrasting CVD and cancer.