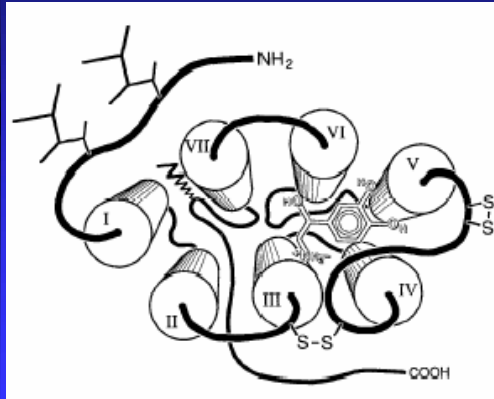


# Analysis of intracellular parts of the $\beta$ -adrenergic receptor family



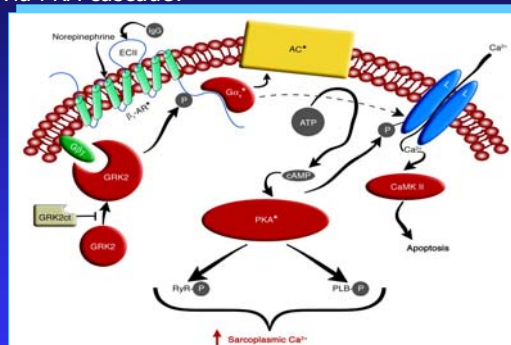
Ref: Ostrowski, J; *Annu.Rev.Pharm.Toxicol.* 1992

Annalisa  
Emanuela  
Mark  
Petrine  
Tibor

EMBO course 2004

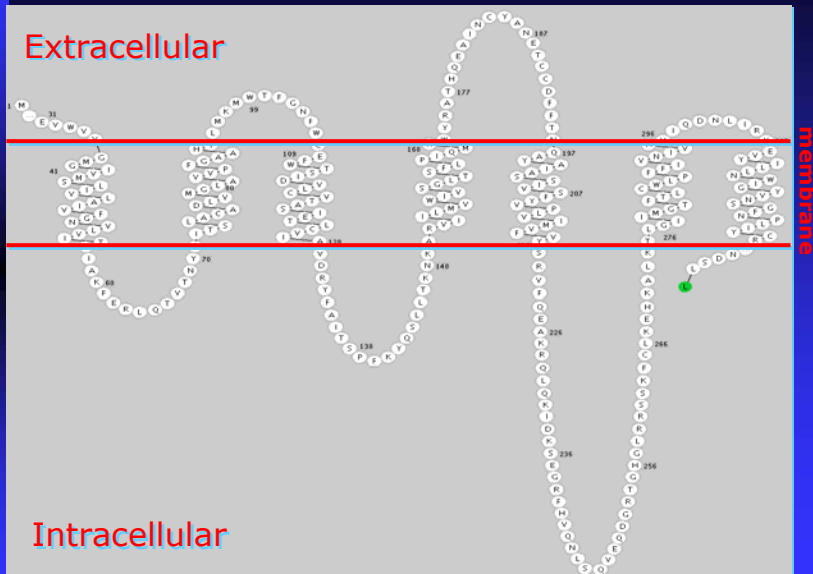
## Properties of $\beta$ adrenoreceptors

- Belong to the family of G-protein coupled receptors
- Three subtypes have been distinguished: **beta1**, **beta2**, and **beta3**
- Signalling via PKA cascade:



	$\beta 1$	$\beta 2$	$\beta 3$
Tissue expression	heart	Skeletal muscle, lung, pancreas	Adipose tissue
function	Inotropic effect	Glycogenolysis, bronchodilatation/relaxation, insulin secretion	Regulation of lipid metabolism

## Topology of the $\beta_2$ adrenoreceptor



GPCRDB: Snake-like view of B2AR\_HUMAN

## Aim of the project

Analyze and compare intracellular domains of the  $\beta$  adrenoreceptors family members :

- Sequence comparison
- Phosphorylation sites
- protein interaction sites

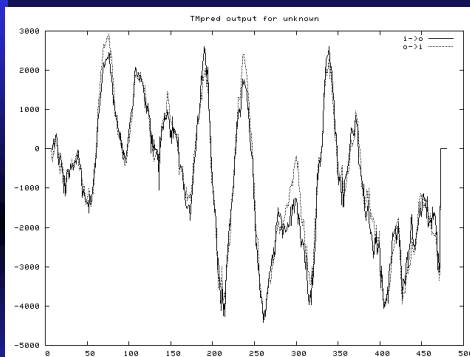


Compare functional consequences

Next slide please!

## Predicting TM segments (hydropathicity analysis)

[www.ch.embnet.org/software/TMPRED\\_form.html](http://www.ch.embnet.org/software/TMPRED_form.html)



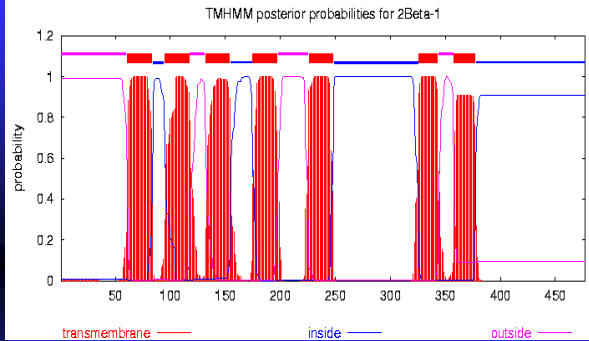
**7 strong transmembrane helices  
total score : 14920**

#	from	to	length	score	orientation
1	63	87	(25)	2944	outside-inside
2	99	125	(27)	2069	inside -outside
3	137	158	(22)	1453	outside-inside
4	181	200	(20)	2601	inside -outside
5	227	249	(23)	2414	outside-inside
6	329	350	(22)	2596	inside -outside
7	362	384	(23)	843	outside-inside

$\beta_2$  adrenoceptor

# Predicting TM segments in GPCRs (Hidden Markov Model) [www.cbs.dtu.dk](http://www.cbs.dtu.dk)

β<sub>2</sub> adrenoreceptor



# 2Beta-1 Exp number, first 60 AAs: 0.60702  
# 2Beta-1 Total prob of N-in: 0.00955

2Beta-1	TMHMM2.0	outside	1	60
2Beta-1	TMHMM2.0	TMhelix	61	83
2Beta-1	TMHMM2.0	inside	84	94
2Beta-1	TMHMM2.0	TMhelix	95	117
2Beta-1	TMHMM2.0	outside	118	131
2Beta-1	TMHMM2.0	TMhelix	132	154
2Beta-1	TMHMM2.0	inside	155	174
2Beta-1	TMHMM2.0	TMhelix	175	197
2Beta-1	TMHMM2.0	outside	198	225
2Beta-1	TMHMM2.0	TMhelix	226	248
2Beta-1	TMHMM2.0	inside	249	325
2Beta-1	TMHMM2.0	TMhelix	326	343
2Beta-1	TMHMM2.0	outside	344	357
2Beta-1	TMHMM2.0	TMhelix	358	377
2Beta-1	TMHMM2.0	inside	378	477

## MSA of adrenergic receptor subtypes

β<sub>1</sub> β<sub>2</sub> β<sub>3</sub>

```

2Beta-1 1 MGAGVVLGSEBENLSSRAEELGAAATAAELEVAEPFAPLSEASEPEPLSQQWRAG
3Beta-3 1 -----MRFHEHENSEPASEEEL-----LPLAARNANLSEEGVP-----EFA
1Beta-2 1 -----MGCSENSIFLLAEL-----EELRSHDHDVQQR-----DEVWVY

2Beta-1 61 MGLMALIVLAVGNVIVIVAIKTRIQTLNLEP SLSRDLVMGLLVVDFGARR
3Beta-3 39 MGLMALIVLAVGNVIVIVIAEERIQTMNVEITSLRDLVMGLLVVDFGARR
1Beta-2 36 MGLMALIVLAVGNVIVIVIAEERIRLQVTVVITSLRDLVMGLLVVDFGARR

2Beta-1 120 LMRWRENSFCELWTSVDVLCVTSIETLCLVIA DRYLAITSPFRYQSLLTRRARLW
3Beta-3 99 LMRWRENSFCELWTSVDVLCVTSIETLCLVIA DRYLAITSPFRYQSLLTRRARLW
1Beta-2 95 LMRWRENSFCELWTSVDVLCVTSIETLCLVIA DRYLAITSPFRYQSLLTRRARLW

2Beta-1 180 LVTALSAIVSFLPIMHWWRARSLRARCYNLECCDFINLRYAIASSVSVFVPL
3Beta-3 159 LVTALSAIVSFLPIMHWWRARSLRARCYNLECCDFINLRYAIASSVSVFVPL
1Beta-2 155 LVTALSAIVSFLPIMHWWRARSLRARCYNLECCDFINLRYAIASSVSVFVPL

2Beta-1 239 LMEFVPERVFEARQVKRIDLERFKGRAPFESSEVPAPAVLGFPEYDAFA
3Beta-3 219 LMEFVPERVFEARQLRLEGGGRFPDPPSFPESSEVPAPAVLGFPEYDAFA
1Beta-2 214 LMEFVPERVFEARQLRLEGGGRFVDEGRFVWVNSVNEG-----DGLFGG

2Beta-1 299 RRPASRLVLRKALTKLGIIMCTFLCWLFFLANVVEAFHRDELVDNR
3Beta-3 277 RRPASRLVLRKALTKLGIIMCTFLCWLFFLANVVEAFGGPSVVEGFP
1Beta-2 258 RRPASRLVLRKALTKLGIIMCTFLCWLFFLANVVEAFVQDNLNRKE

2Beta-1 358 FSNLGYANSRNPFIYCRSPDPERAFQELLCLEARRRGGGSEFSSAR
3Beta-3 327 FSNLGYANSRNPFIYCRSPDPERAFQELLCLEARRRGGGSEFSSAR
1Beta-2 307 FSNLGYANSRNPFIYCRSPDPERAFQELLCLEARRRGGGSEFSSAR

2Beta-1 418 EGFSEGADLWVWVWVRLERWGGNGALNMRDAPRNGANSV
3Beta-3 377 EGFSEGAAARWVWVWVRLERWGGNGALNMRDAPRNGANSV
1Beta-2 351 EGFSEGVVGEWVWVWVRLERWGGNGALNMRDAPRNGANSV

2Beta-1 478 ---
3Beta-3 ---
1Beta-2 411 EL
    
```

ICL1

ICL2

ICL3

C-term

# Motif search using Scansite

[http://scansite.mit.edu/motifscan\\_seq.phtml](http://scansite.mit.edu/motifscan_seq.phtml)

$\beta_2$

**Basophilic serine-threonine kinase group Protein kinase A**

S30 0.3259 0.153 LLCLRRSSLKAYGNG 1.343  
C-term

$\beta_3$

**Src homology 3 group (SH3) Intersectin SH3A**  
P72 0.5287 0.104% PEESPPAPSRSLAPA 1.947  
ICL 3

$\beta_1$

**Src homology 3 group (SH3) Acidophilic serine-threonine kinase group (GSK3 kinase) Proline-dependent serine-threonine kinase group (Erk1 kinase) Kinase binding site group Erk D-domain MAPK**

# Motif search using Scansite

[http://scansite.mit.edu/motifscan\\_seq.phtml](http://scansite.mit.edu/motifscan_seq.phtml)

$\beta_1$

**Src homology 3 group (SH3)**  
**Intersectin SH3A** Gene Card ILSN

Site	Score	Percentile	Sequence	S	A
P12	0.3131	0.006 %	APAPPPGPPRPA	2	53

**Crk SH3** Gene Card CRK

Site	Score	Percentile	Sequence	S	A
P11	0.3591	0.026 %	PVPAPAPPPGPPR	1	31

**PLC $\gamma$  SH3** Gene Card PLCG1

Site	Score	Percentile	Sequence	S	A
P12	0.4815	0.069 %	PAPPPGPPRPA	1	65

**Cortactin SH3** Gene Card ICLS1

Site	Score	Percentile	Sequence	S	A
P12	0.5269	0.154 %	APAPPPGPPRPA	2	53

**Cbl-Associated protein C-SH3** Gene Card N/A

Site	Score	Percentile	Sequence	S	A
P12	0.6156	0.173 %	APAPPPGPPRPA	2	53

**Acidophilic serine/threonine kinase group (Acid\_ST\_kin)**  
**GSK3 Kinase** Gene Card GSK3A

Site	Score	Percentile	Sequence	S	A
S10	0.326	0.008 %	GGPARPPSPSPVP	2	235

**GSK3 Kinase** Gene Card GSK3A

Site	Score	Percentile	Sequence	S	A
S11	0.350	0.014 %	RPPSPSPVPAPAP	2	112

**Proline-dependent serine/threonine kinase group (Pro\_ST\_kin)**  
**Erk1 Kinase** Gene Card EFHE2

Site	Score	Percentile	Sequence	S	A
S10	0.411	0.077 %	PARPPSPSPVPAP	2	203

**Erk1 Kinase** Gene Card EFHE2

Site	Score	Percentile	Sequence	S	A
S11	0.423	0.103 %	RPPSPSPVPAPAP	2	112

**Erk1 Kinase** Gene Card EFHE2

Site	Score	Percentile	Sequence	S	A
S10	0.423	0.103 %	GGPARPPSPSPVP	2	235

**Kinase binding site group (Kin\_bind)**  
**Erk D-domain** Gene Card MAPK1

Site	Score	Percentile	Sequence	S	A
V14	0.566	0.144 %	KRRPSRLVALREOKA	7	84

## Motif search of $\beta_1$ using Scansite [http://scansite.mit.edu/motifscan\\_seq.phtml](http://scansite.mit.edu/motifscan_seq.phtml)

ICL3

TVWAISALVSFLPILMHWWRAESDEARRCYREAQKQVKKIDSCERF  
LGGPARPPSPSPVVPAPAPP~~PP~~RPAAAAATAPLANGRAGKRRPSR  
LVALREQKALKTLGI

**GSK3 kinase**

**Erk1 kinase**

**Crk SH3**

**Cb1-associated protein (C-SH 3)**

**Contactin SH3**

**Intersectin SH3 (also found in beta3 AR)**

**PLC $\gamma$  SH3**

**Erk-D domain**

## Phosphorylation sites in C-termini using Prosite <http://www.expasy.org/prosite/>

$\beta_2$

↪ Also found in motifscan – hurrah!

CRSPDFRIAFQELLCL[RRS[S]LK]AYGNGYSSNGNTGEQSG  
YHVEQEKENKLLCEDLPGTEDFVGHQGTVPDNDISQGRN  
C[STND]SLL

**CK2**

**PKA**

**PKC**

$\beta_1$

CRSPDFRKAQGLLCCARRAARRRHA~~THGDR~~PRASGCLAR  
PGPPSPGAA~~SDDD~~DDDDVVGATPPARLLEPWAGCNGGAA  
ADSD[S[SLD]E]PCRPGFAS

$\beta_3$

NWLGYANSFAFNLIYCRSPDFRSFRLLCRCGRRLPPECAAAARPALF  
PSGVPAARSSPAQPRLCQRLDGASWGV

## Conclusions

-Important to try different programs and algorithms

### TM prediction:

- Sosui predicted 6TMs for both the beta2 AR and rhodopsin (should not be used for 7TM receptors???)
- TMPRED (hydropathicity) and TM HMM gave expected and correlating results

### Prediction of protein-protein interaction/phosphorylation sites:

- only a few sites are consistently found (PKA for beta2)
  - should be found in beta1 as well according to litt.
  - more time is required to compare all findings with litt!!

**REMEMBER: predictions are just predictions!!**



'Mr. Kramer, may I be excused? My brain is full.'