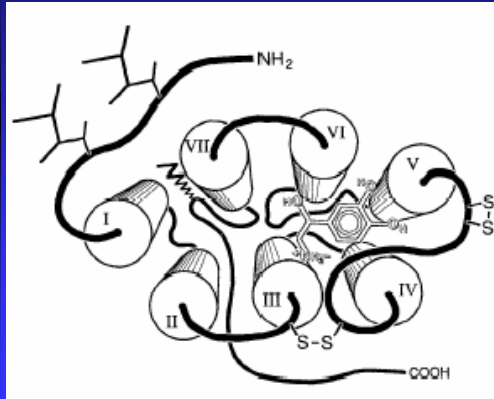


Analysis of intracellular parts of the β -adrenergic receptor family



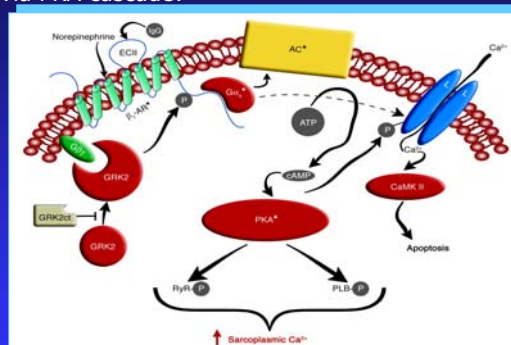
Ref: Ostrowski, J; *Annu.Rev.Pharm.Toxicol.* 1992

Annalisa
Emanuela
Mark
Petrine
Tibor

EMBO course 2004

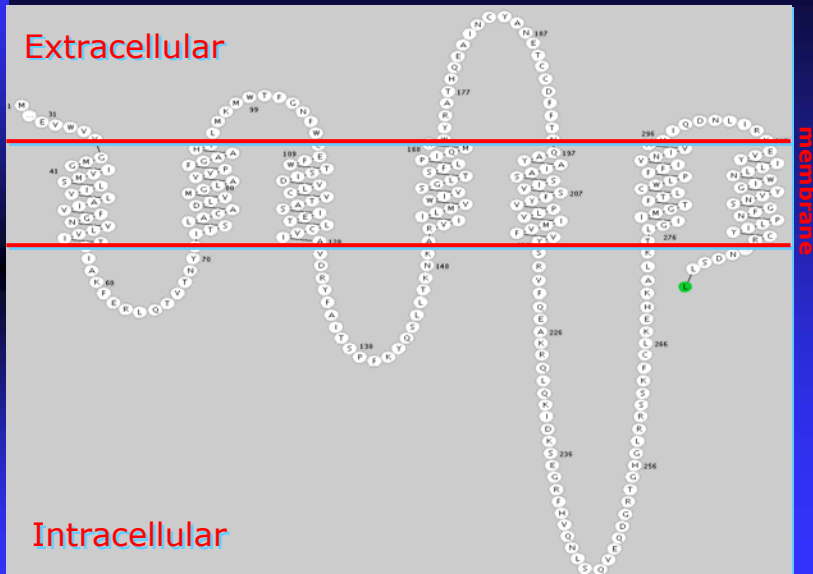
Properties of β adrenoreceptors

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



	β_1	β_2	β_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 β_2 adrenoreceptor



GPCRDB: Snake-like view of B2AR_HUMAN

Aim of the project

Analyze and compare intracellular domains of the β adrenoreceptors family members :

- Sequence comparison
- Phosphorylation sites
- protein interaction sites

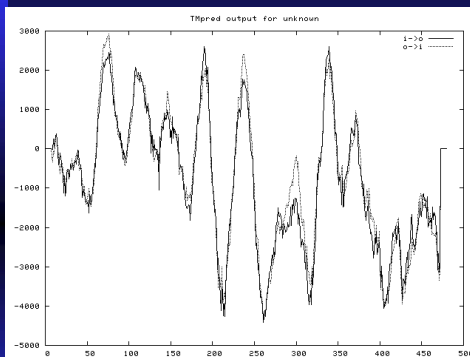


Compare functional consequences

Next slide please!

Predicting TM segments (hydropathicity analysis)

www.ch.embnet.org/software/TMPRED_form.html



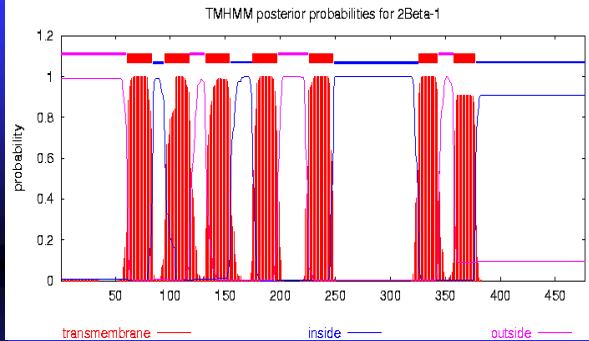
**7 strong transmembrane helices
total score : 14920**

#	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

β_2 adrenoreceptor

Predicting TM segments in GPCRs (Hidden Markov Model) www.cbs.dtu.dk

β₂ 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

β₁ β₂ β₃

```

2Beta-1 1 MGAGVVLGSEBENLSSRAEELGAAATAAELEVAEPFAPLSEASEPEPLSQQWRAG
3Beta-3 1 -----MRFHEHENSEPASEEEL-----LPLAARNANLSEEGVP-----EFA
1Beta-2 1 -----MGCSENSIFLLAEL-----EELRSHDHVQQR-----DEVVTVG

2Beta-1 61 MGLMALIVLAVGNVIVIVAIKTRIQTLNLEP SLSRDLVMGLLVVDFGARR
3Beta-3 39 MGLMALIVLAVGNVIVIVAIKTRIQTMNVE TSLRDLVMGLLVVDFGARR
1Beta-2 36 MGLMALIVLAVGNVIVIVAIKTRIQTLNLEP SLSRDLVMGLLVVDFGARR

2Beta-1 120 LMRKREKSSICELWTSVDVLCVTSIETLCLVIA DRYLAITSPFRYQSLLTR RAR LW
3Beta-3 99 LMRKREKSSICELWTSVDVLCVTSIETLCLVIA DRYLAITSPFRYQSLLTR RAR LW
1Beta-2 95 LMRKREKSSICELWTSVDVLCVTSIETLCLVIA DRYLAITSPFRYQSLLTR RAR LW

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

2Beta-1 239 LMEFVPERVFEA KQVKRID E RPKGGRAPFES S PAPAUIGPEYDA A
3Beta-3 219 LMEFVPERVFEA RQLR LGGGGRFPDPPSFPES S PAPAUIGPEYDA A
1Beta-2 214 LMEFVPERVFEA RQLR KID E RGRNWNISVNEG-----DGLGIG

2Beta-1 299 RRPASRLV LRE KALKTLGIIMCTFLCWLFFLANVVFHR-ELEVDR
3Beta-3 277 RRPARLL LREHRA ELICIMCTFTLCWLFFLANVVFHGGPSVIVGFP
1Beta-2 258 RRPASRLV LRE KALKTLGIIMCTFTLCWLFFLANVVFHQQD-NLRKE

2Beta-1 358 FSNLGYANSRNPFIYCRSPDPERAPQ ILC RARRRGGGSEF S IAR
3Beta-3 327 FSNLGYANSRNPFIYCRSPDPERAPQ ILC RARRRGGGSEF S IAR
1Beta-2 307 FSNLGYANSRNPFIYCRSPDPERAPQ ILC RARRRGGGSEF S IAR

2Beta-1 418 EG FSGADLVVWVWVRLERWAGNGCAVWNRDAPRNGANS S
3Beta-3 377 EAFPSGVFAARGLRGLRSGPQRLCQLGARGGV-----
1Beta-2 351 ENLYS YGVGEGRWVYGRKRNILICEIICRHDVCGRQVPEENITDSGANGC TNG

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

β_2

Basophilic serine-threonine kinase group Protein kinase A

S30 0.3259 0.153 LLCLRRSSLKAYGNG 1.343
C-term

β_3

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

β_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

β_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 γ 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	2.35

GSK3 Kinase Gene Card GSK3A

Site	Score	Percentile	Sequence	S	A
S11	0.350	0.014 %	RPPSPSPVPAPAP	2	1.12

Proline-dependent serine/threonine kinase group (Pro_ST_kin)

Erk1 Kinase Gene Card EPHB2

Site	Score	Percentile	Sequence	S	A
S10	0.411	0.077 %	PARPPSPSPVPAP	2	2.03

Erk1 Kinase Gene Card EPHB2

Site	Score	Percentile	Sequence	S	A
S11	0.423	0.103 %	RPPSPSPVPAPAP	2	1.12

Erk1 Kinase Gene Card EPHB2

Site	Score	Percentile	Sequence	S	A
S10	0.423	0.103 %	GGPARPPSPSPVP	2	2.35

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	8.44

Motif search of β_1 using Scansite http://scansite.mit.edu/motifscan_seq.phtml

ICL3

TVWAISALVSFLPILMHWWRAESDEARRCYREAQKQVKKIDSCERF
LGGPARPPSPSPVVPAPAPP~~PGPP~~RPAAAAATAPLANGRAGKRRPSR
LVALREQKALKTLGI

GSK3 kinase

Erk1 kinase

Crk SH3

Cb1-associated protein (C-SH 3)

Contactin SH3

Intersectin SH3 (also found in beta3 AR)

PLC γ SH3

Erk-D domain

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

β_2

↪ Also found in motifscan – hurrah!

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

CK2

PKA

PKC

β_1

CRSPDFRKAQGLLCCARRAARRRHA~~THGDR~~PRASGCLAR
PGPPSPGAA~~SDDDD~~DDVVGGATPPARLLEPWAGCNGGAA
ADSD[S[SLD]E]PCRPGFAS

β_3

NWLGYANSAFNLIYCRSPDFRSFRLLCRCGRRLPPECAAAARPALF
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.'