Methodological Approaches To Study Receptors

Lecture #2

Determining the Stoichiometry of Receptors

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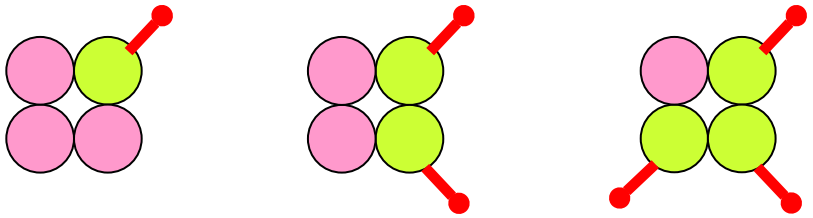
Determining the Stoichiometry of Receptors

1. Selective Tagging

Mutagenesis or Selective Drugs



Subunit Copy Number

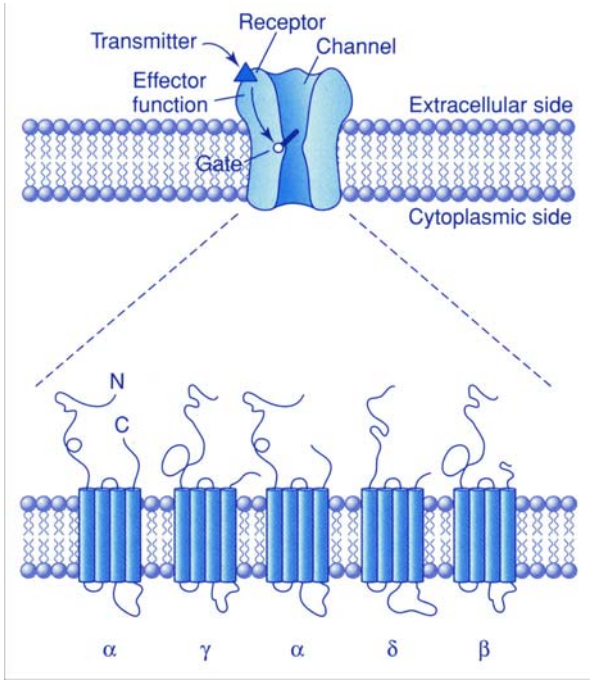
2. Constraining Stoichiometry

Covalently-Coupled Subunits

Relative Subunit Position

1. Selective Tagging-Mutagenesis

$GABA_A$ Receptor Stoichiometry



4 Different Classes of Subunit

α, β, γ and δ

Multiple Isoforms for Each Subunit

$\alpha_{1-6}\,\beta_{1-3}\,\gamma_{1-3}\,\delta_1$

Members of Superfamily of Channels

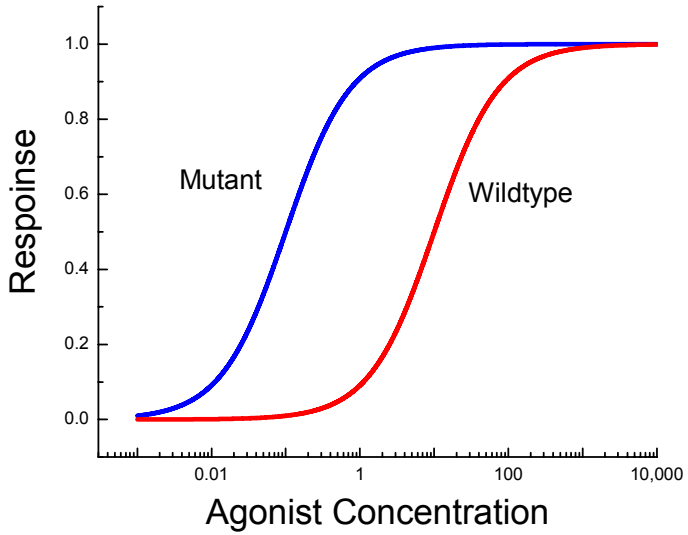
Others include 5-HT & nACh receptors

Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

1. Selective Tagging-Mutagenesis

$GABA_A$ Receptor Stoichiometry

Leu to Ser Mutation increases agonist sensitivity



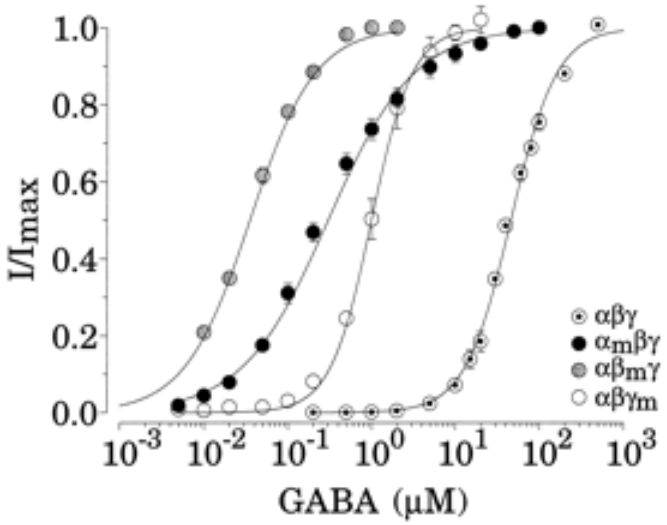
Conserved Leucine Residue Increases Agonist Sensitivity

$\alpha 1(L_{263}S)$	CAA GGT TGT CAT GGT **ACT** AAC GGT CGT CAC TCC
$\beta 2(L_{259}S)$	GAT TGT GGT CAT CGT **ACT** GAC AGT TGT AAT TCC
$\gamma 2(L_{274}S)$	GAG AGT GGT CAT CGT **ACT** GAC AGT CGT GAT TCC

Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

1. Selective Tagging-Mutagenesis

$GABA_A$ Receptor Stoichiometry



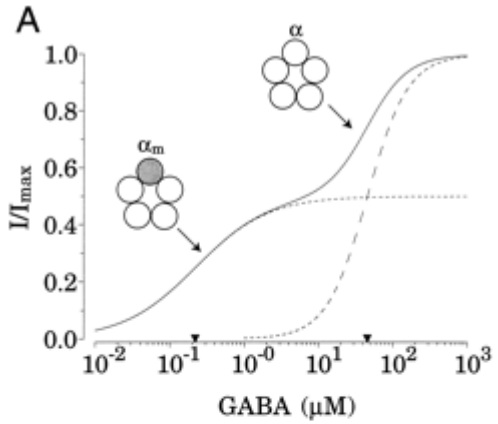
Subunit	EC50
$\alpha\beta\gamma$	46 μM
$\alpha\beta\gamma_m$	1 μM
$\alpha_m\beta\gamma$	0.3 μM
$\alpha\beta_m\gamma$	0.03 μM

Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

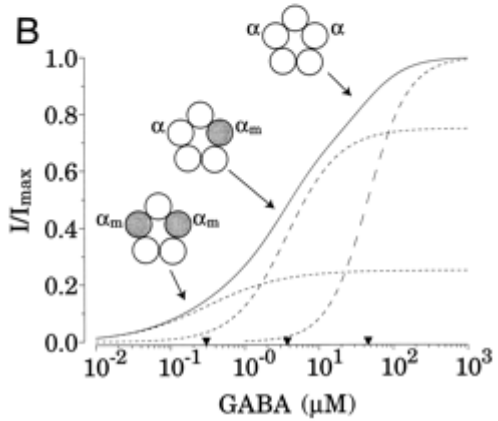
1. Selective Tagging-Mutagenesis

$GABA_A$ Receptor Stoichiometry

Theory: how many α subunits?



For a single α subunit = 2 components

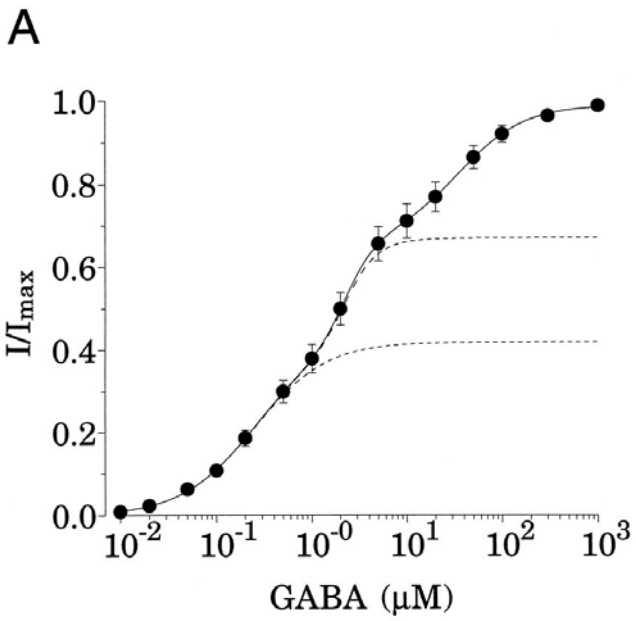


For two α subunit = 3 components

Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

1. Selective Tagging-Mutagenesis

$GABA_A$ Receptor Stoichiometry



Experiment: how many α subunits?

D/R curve has 3 components

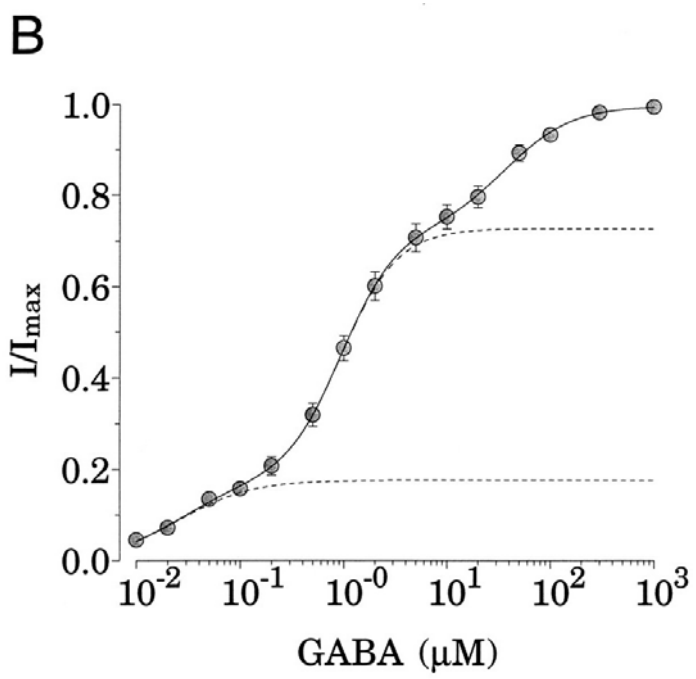
EC_{50} values, 0.26, 2.3 & 36 μM

Therefore, mature $GABA_A$ receptors contain two α subunits

Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

1. Selective Tagging-Mutagenesis

$GABA_A$ Receptor Stoichiometry



Experiment: how many β subunits?

D/R curve has 3 components

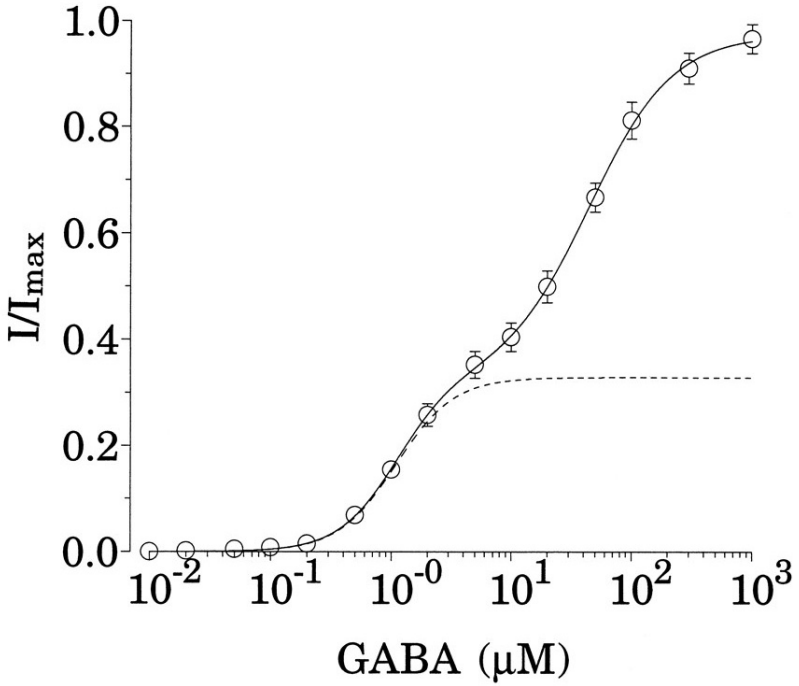
EC_{50} values, 0.025, 0.94 & 39 μM

Therefore, mature $GABA_A$ receptors contain two β subunits

Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

1. Selective Tagging-Mutagenesis

$GABA_A$ Receptor Stoichiometry



Experiment: how many γ subunits?

D/R curve has 2 components

EC_{50} values, 1 & 41 μM

Therefore, mature $GABA_A$ receptors contain one γ subunit

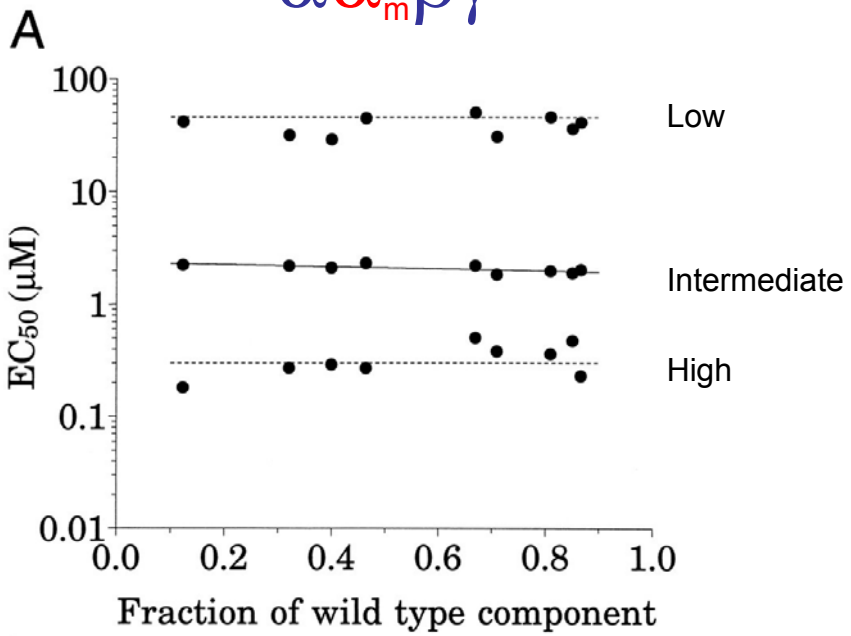
Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

1. Selective Tagging-Mutagenesis

$GABA_A$ Receptor Stoichiometry

Question: Does wildtype/mutant subunit fraction affect outcome?

$\alpha\alpha_m\beta\gamma$



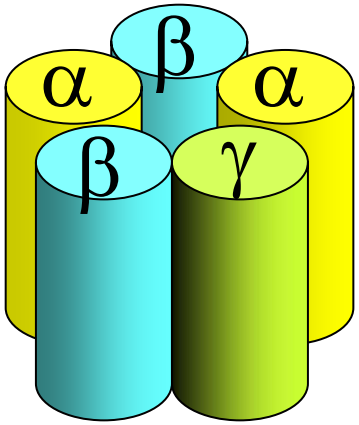
All D/R curves have 3 components

Similar findings for β and γ subunits

Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

$GABA_A$ Receptor Stoichiometry

Interpretation



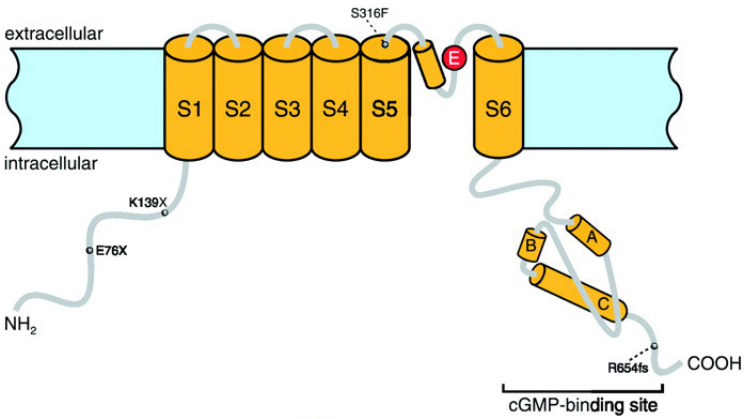
Mature $GABA_A$ receptors contain;

2 x α_1 subunits
2 x β_2 subunits
1 x γ_2 subunit

Further reading Chang et al, J. Neurosci (1996) 16, 5415-5424

2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels



Activated by intracellular cyclic nucleotides

cAMP cGMP

Roles in Sensory Transduction

visual or olfactory signaling

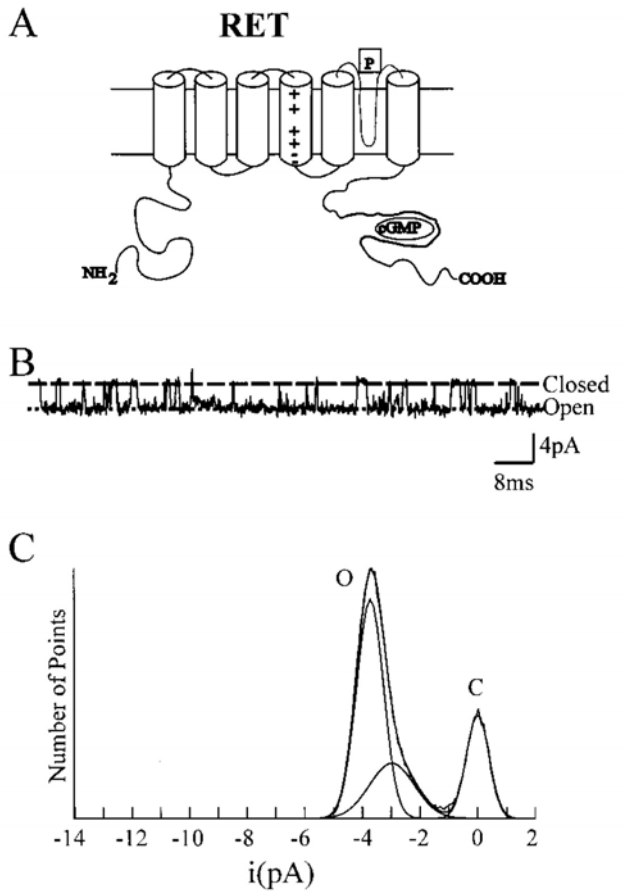
Members of Superfamily of Channels

Others K channels, Glutamate receptors

Further reading Lui et al, Neuron (1996) 16, 983-990

2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels



Experiment: Wildtype α1 RET subunit
(bovine retinal channel)

α1 Homomers

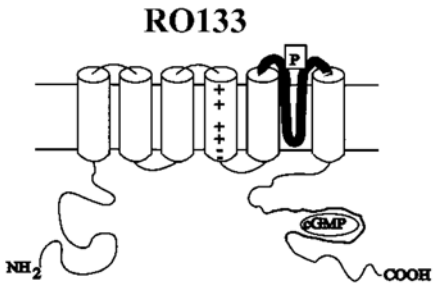
Tetramers or Pentamers?

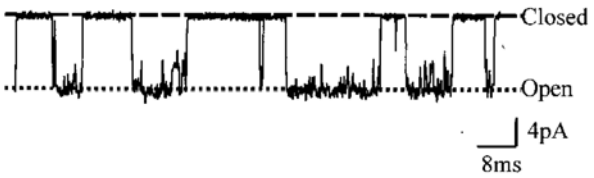
Low channel conductance, 30 pS
Small Pore Diameter, 5.9 Angstroms

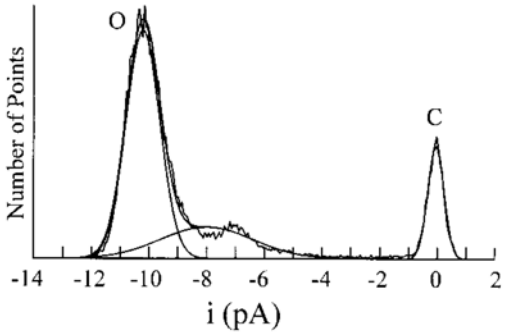
Further reading Lui et al, Neuron (1996) 16, 983-990

2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels







Experiment: Chimera RO133 subunit
(catfish pore region)

RO133 Homomers

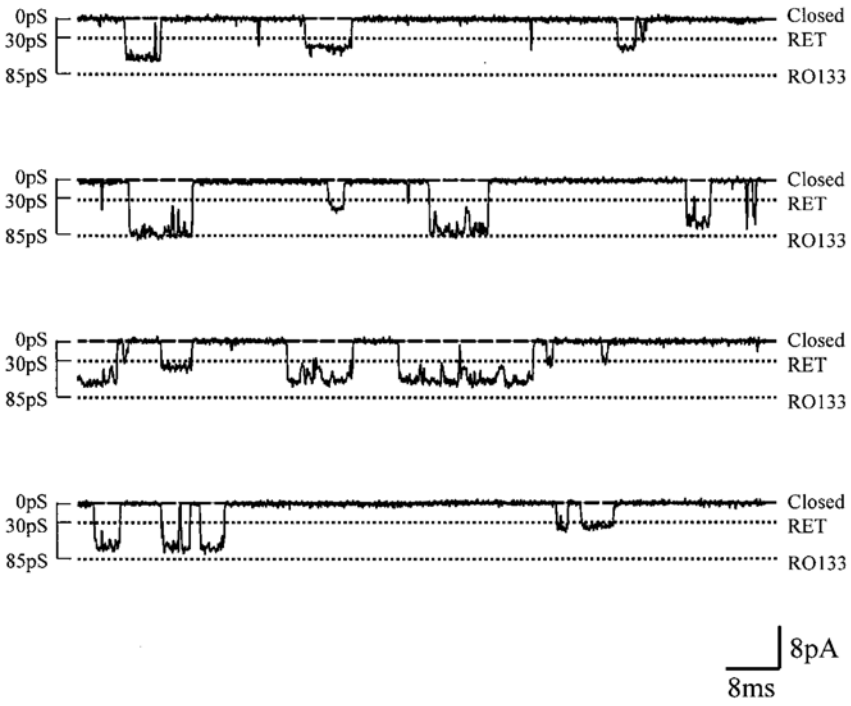
Tetramers or Pentamers?

High channel conductance, 85 pS
Larger Pore Diameter, 6.5 Angstroms

Further reading Lui et al, Neuron (1996) 16, 983-990

2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels



Experiment:
Mixing RET & RO133 subunits

Intermediate conductance levels consistent with heteromeric assemblies

Further reading Lui et al, Neuron (1996) 16, 983-990

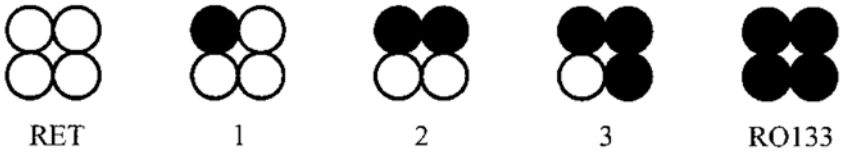
2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels

Theory:

Mixing RET & RO133 subunits

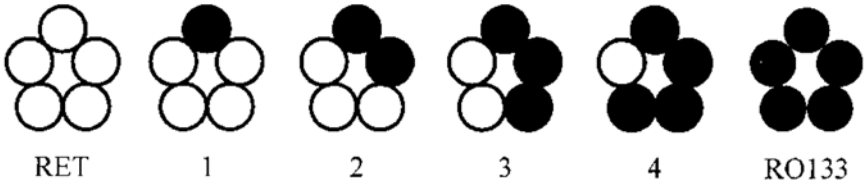
A TETRAMER



Tetramers:

3 Intermediate conductance levels

B PENTAMER



Pentamers:

4 Intermediate conductance levels

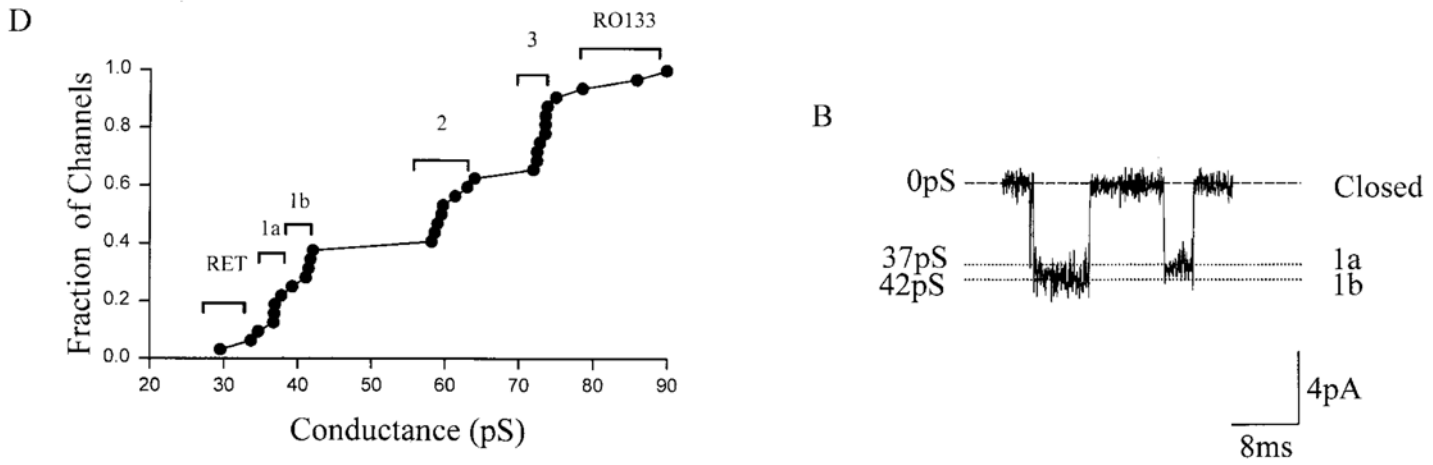
Further reading Lui et al, Neuron (1996) 16, 983-990

2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels

Experiment:

Mixing RET & RO133 subunits



Question

Is there 3 or 4 Intermediate conductance levels?

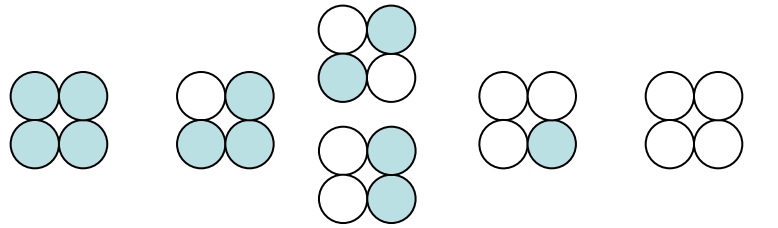
Further reading Lui et al, Neuron (1996) 16, 983-990

2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels

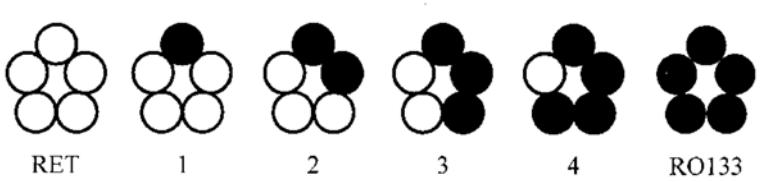
Two Possibilities:

Mixing RET & RO133 subunits



Tetramer:

4 Intermediate conductance levels



Pentamer:

4 Intermediate conductance levels

Further reading Lui et al, Neuron (1996) 16, 983-990

2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels

Experiment:

Is Order of Subunit Assembly Important?

Covalently-Coupled Subunits

The Importance Of The Relative Subunit Position Can Be Determined

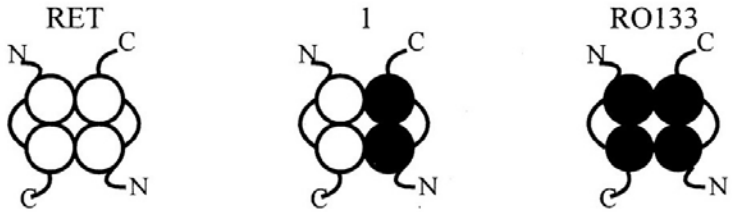
Further reading Lui et al, Neuron (1996) 16, 983-990

2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels

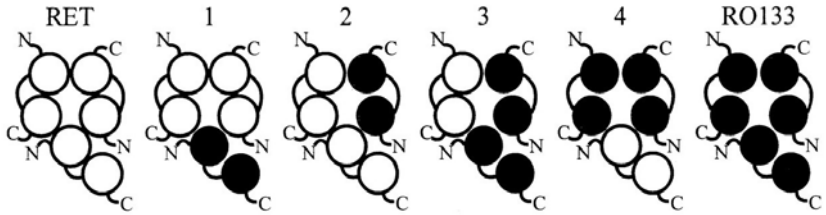
Theory:

Is Order of Subunit Assembly Important?



Tetramer:

1 Intermediate conductance levels



Pentamer:

4 Intermediate conductance levels

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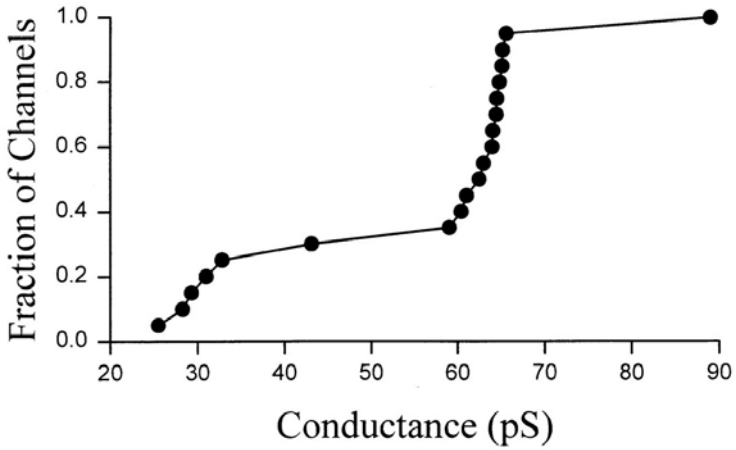
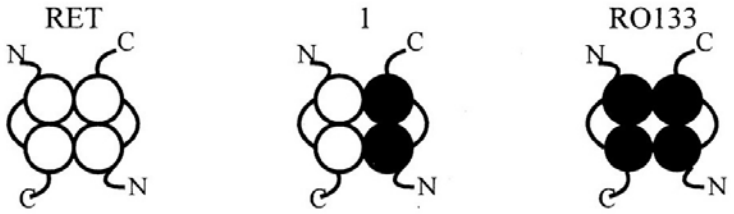
2. Constraining Stoichiometry-Tandem Dimers

Cyclic Nucleotide-Gated Channels

Experiment & Interpretation

Is Order of Subunit Assembly Important?





Tetramer:

1 Intermediate conductance levels

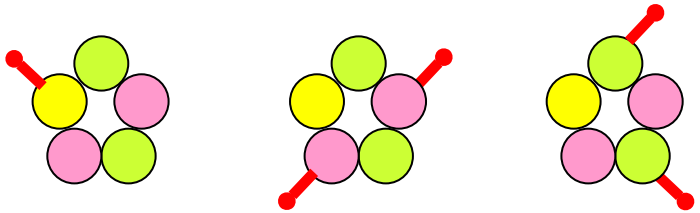
Conclusion: CNG channels assemble as tetramers

Further reading Lui et al, Neuron (1996) 16, 983-990

Determining the Stoichiometry of Receptors

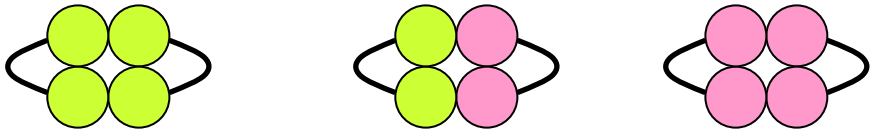
What Have We Learned?

1. Selective Tagging Using Mutagenesis



$GABA_A$ receptors assemble as pentamers

2. Constraining Stoichiometry Using Tandem Dimers



CNG channels assemble as tetramers

Determining the Stoichiometry of Receptors

Further Reading

1. Chang, Y., Wang, R., Barot, S. and Weiss, D.S. (1996) Stoichiometry of a recombinant $GABA_A$ receptor. J. Neurosci, 16, 5415-5424.

2. Liu, D, Tibbs, G.R. and Siegelbaum, S.A. (1996) Subunit stoichiometry of cyclic nucleotide-gated channels and effects of subunit order on channel function. Neuron, 16, 983-990.