Allosteric Model for Ca^{2+} and $InsP_3$ Regulation of $InsP_3R$ Single-channel Gating

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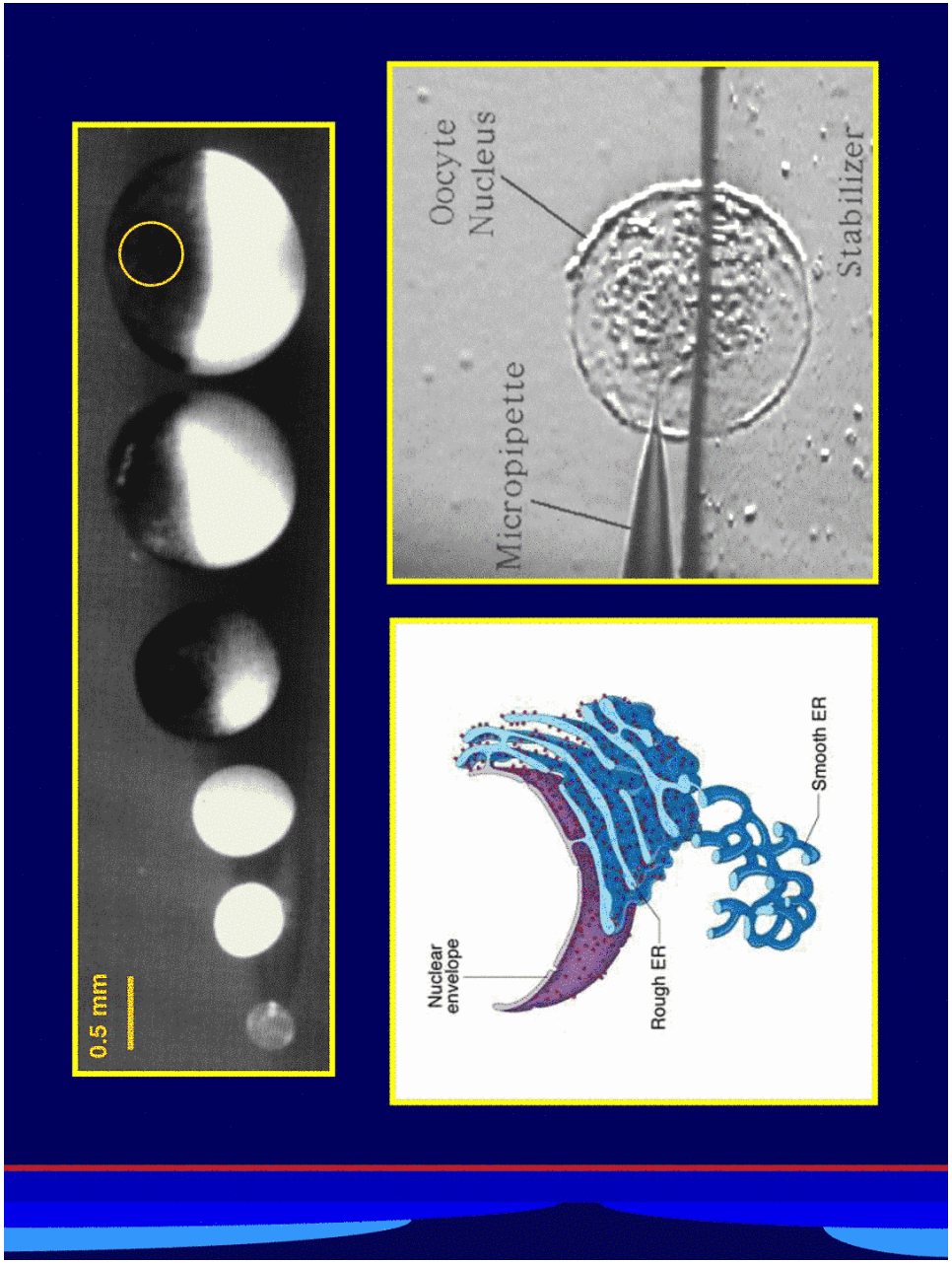
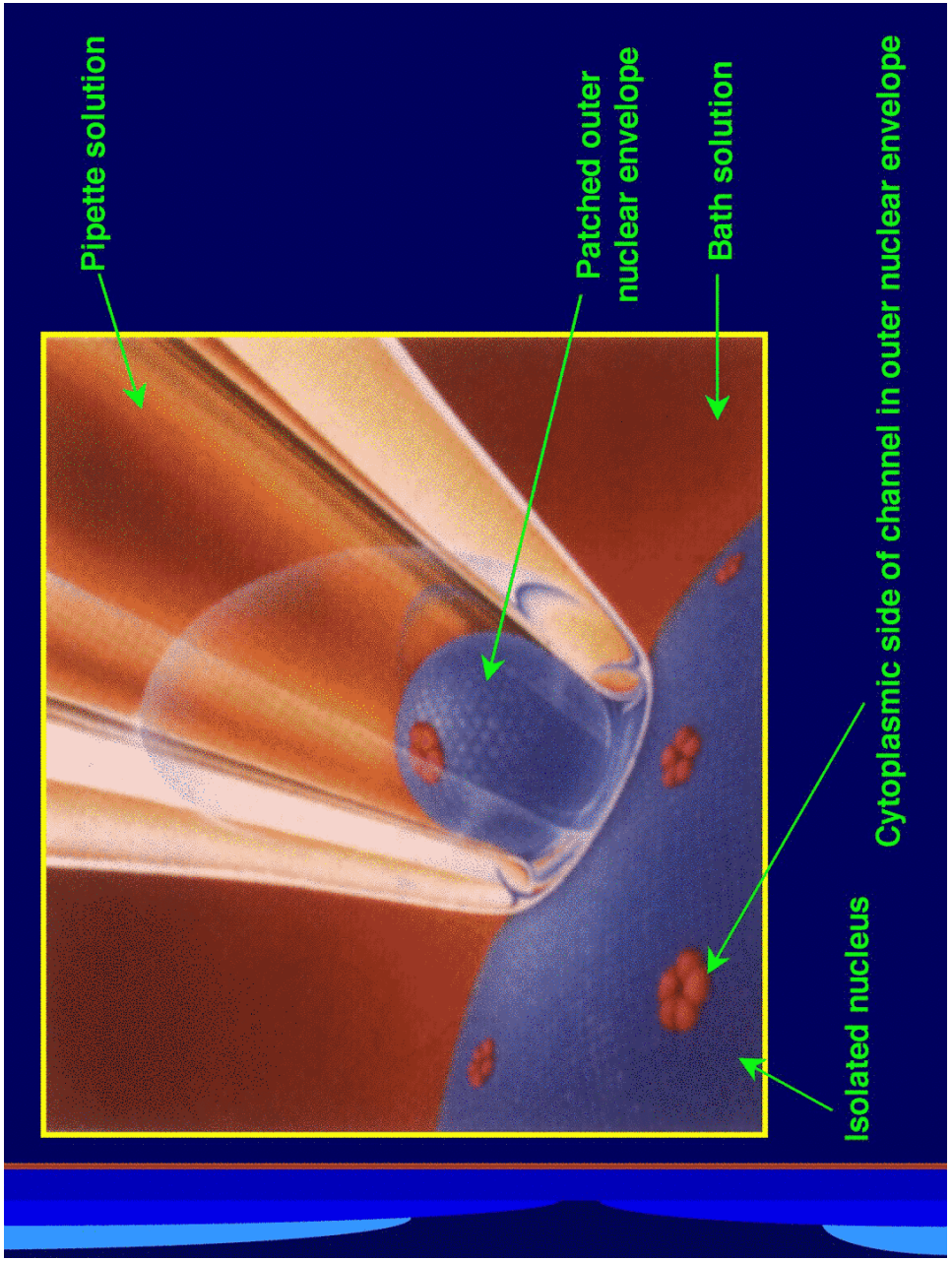
Acknowledgement

- J. Kevin Foskett
- Foskett Lab:
  - » Sean M. J. McBride
  - » Nataliya B. Petrenko
- Frank T. Horrigan

- Background on $InsP_3R$ and Nuclear Patch Clamping
- Experimental observations:
  - (A) Regulation of $InsP_3R$ channel activities by $[Ca^{2+}]_i$
  - (B) Effects of $[InsP_3]$ on $InsP_3R$ channel activities
  - (C) Spontaneous $InsP_3R$ channel activity in the absence of ligand
  - (D) Channel activities of $InsP_3R$ exposed to ultra-low $[Ca^{2+}]$
- Model to account for all experimental observations

- $InsP_3R$ channel is an intracellular Ca^{2+}-release channel (like RyR)
- Found in all cell types studied, localized mostly to the endoplasmic reticulum membrane
- Regulate Ca^{2+} signaling in cell processes like secretion, gene expression, apoptosis and long term depression in nervous system

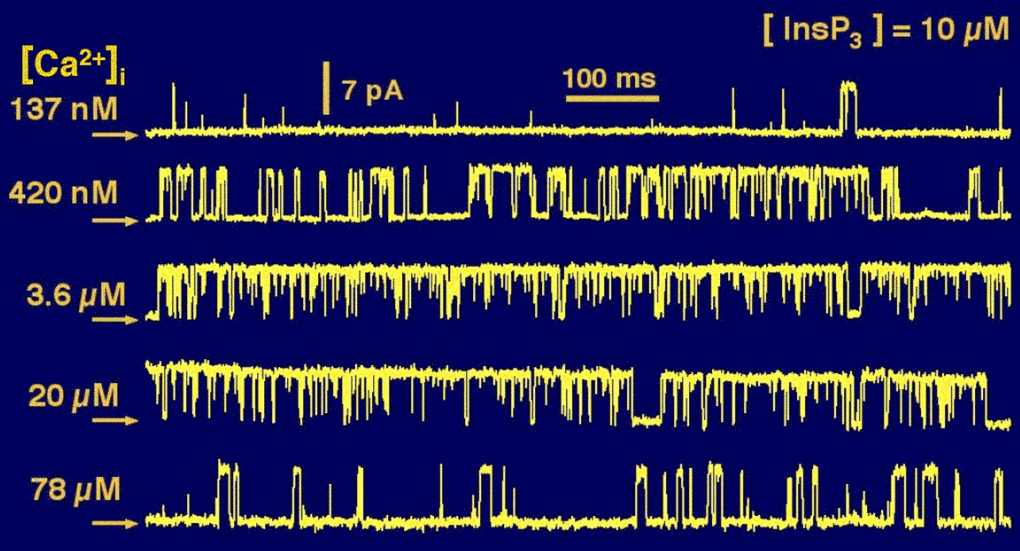

0.5 mm
Oocyte
Nucleus
Micropipette
Stabilizer
Nuclear envelope
Rough ER
Smooth ER


Pipette solution
Patched outer nuclear envelope
Bath solution
Cytoplasmic side of channel in outer nuclear envelope
Isolated nucleus

- Experimental observations:

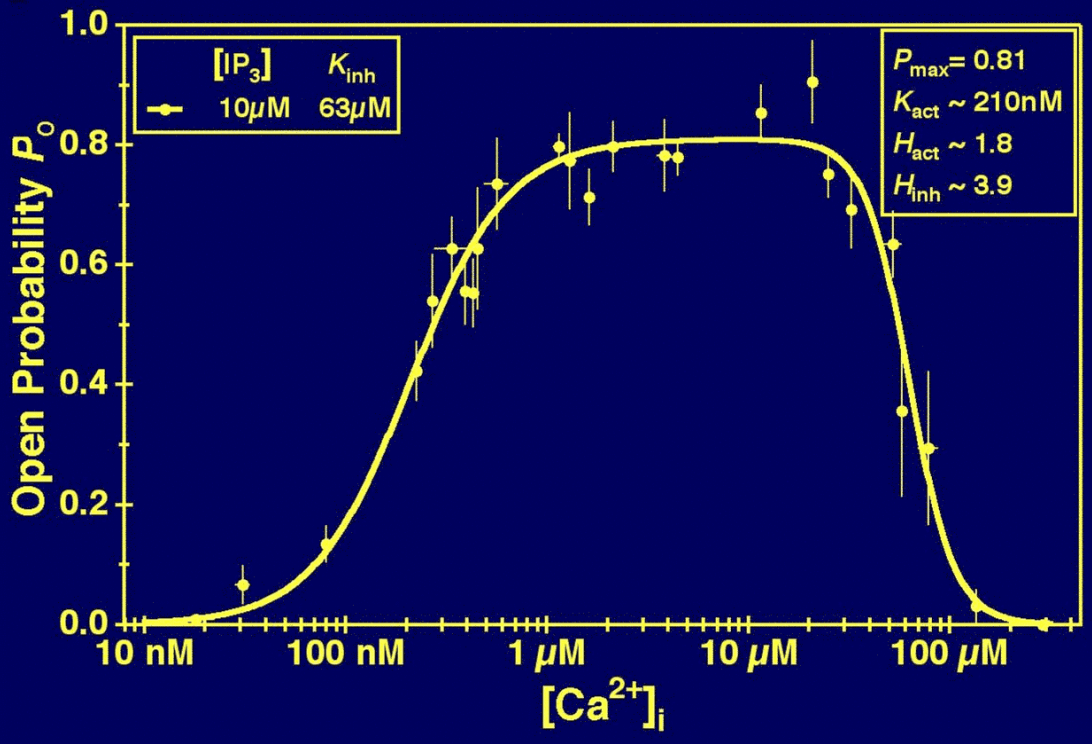
  (A) Regulation of $InsP_3R$ channel activities by $[Ca^{2+}]_i$

$InsP_3R$ channel activity is regulated by $[Ca^{2+}]_i$



Oocyte nuclei isolated directly into bath solution with $[Ca^{2+}] \approx 450$ nM

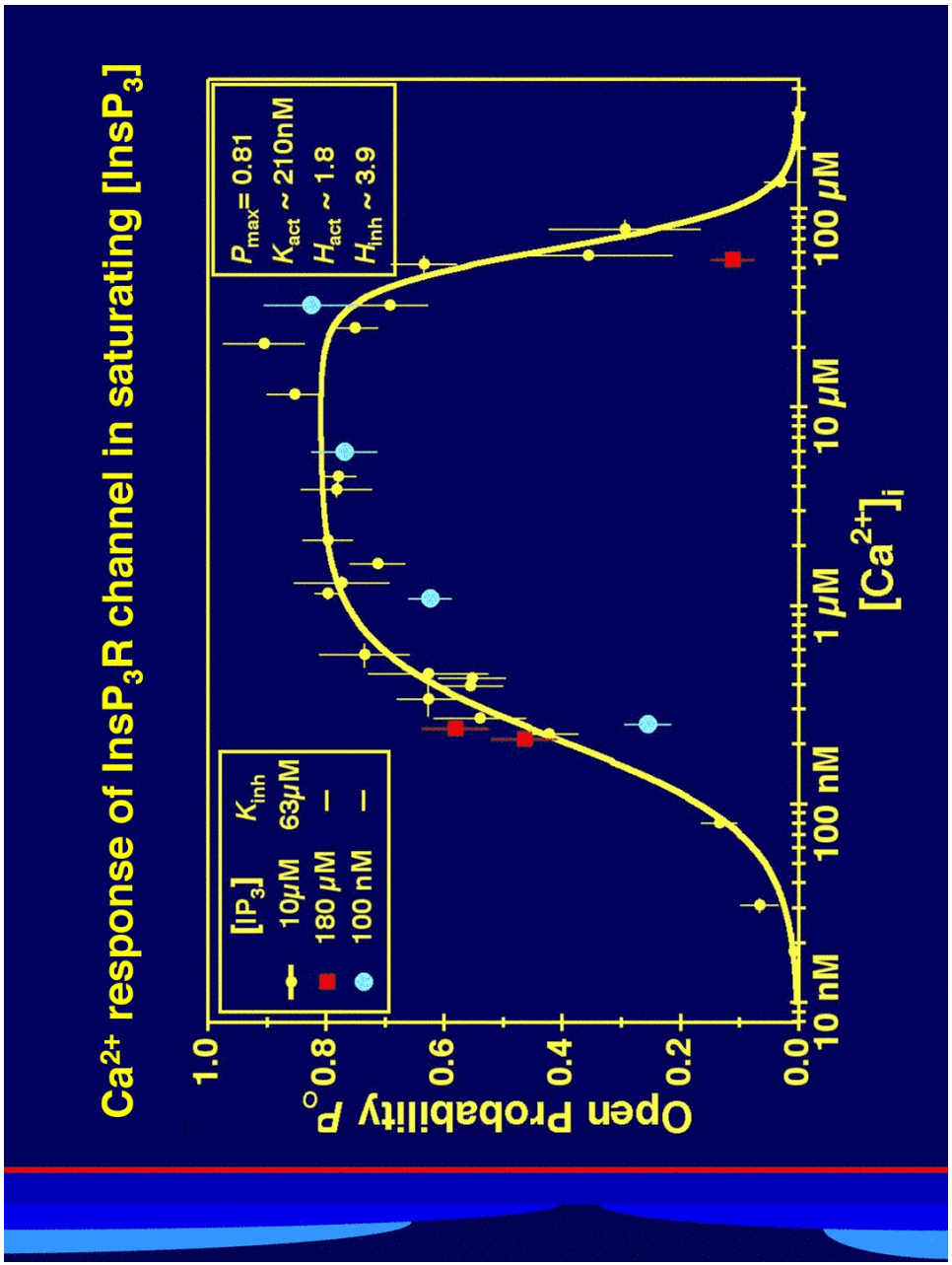
Ca^{2+} response of $InsP_3R$ channel in saturating $[InsP_3]$

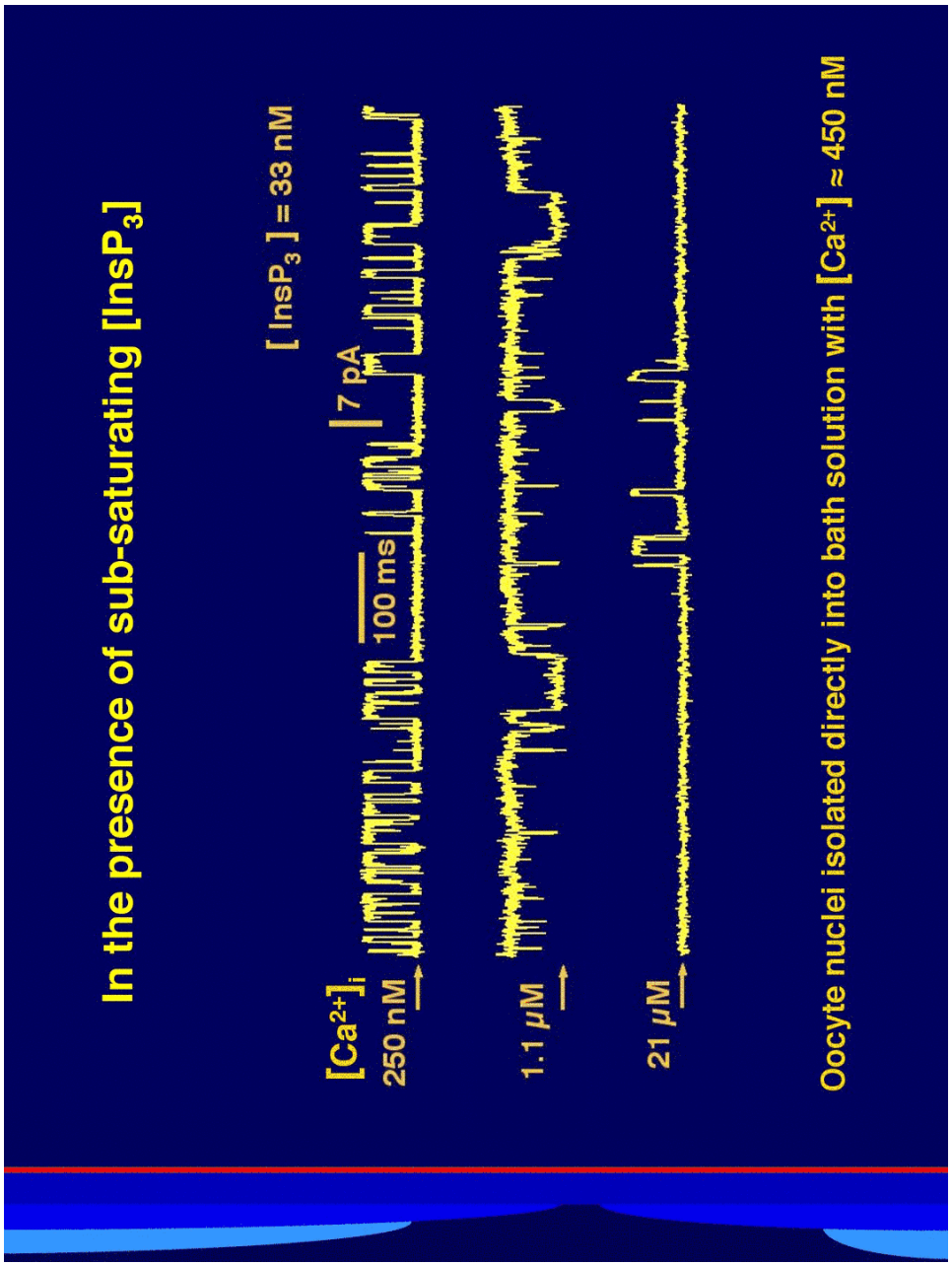


- Experimental observations:

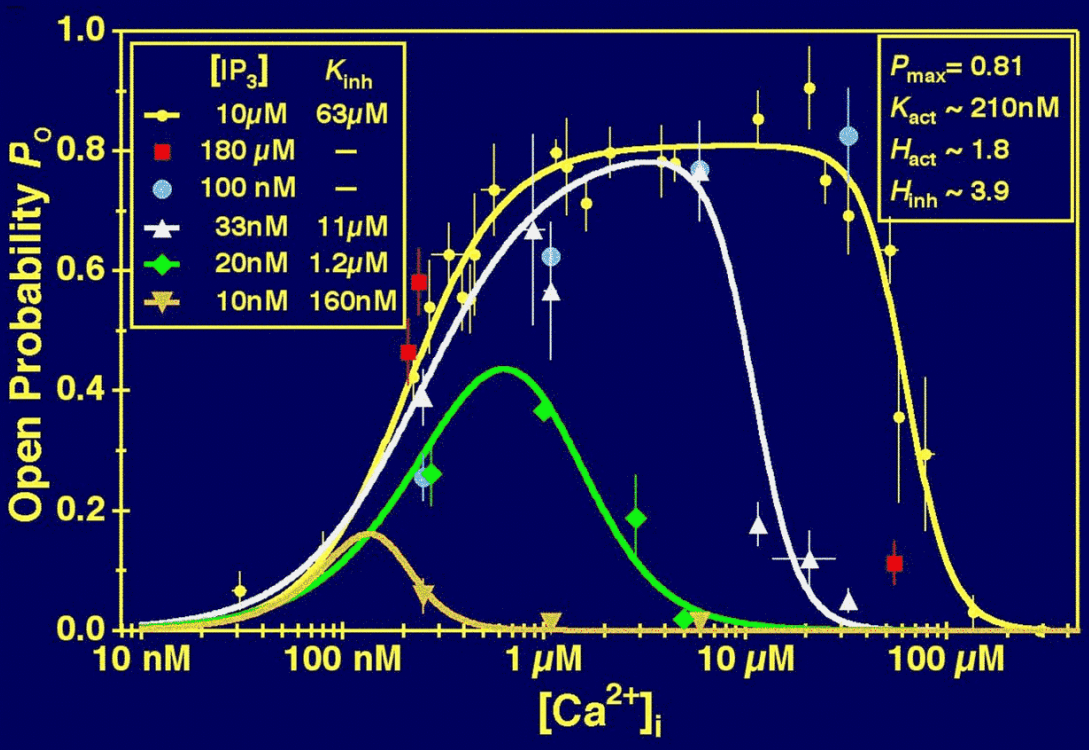
  (A) Regulation of $InsP_3R$ channel activities by $[Ca^{2+}]_i$

  (B) Effects of $[InsP_3]$ on $InsP_3R$ channel activities


Ca2+ response of InsP3R channel in saturating [InsP3]
Open Probability Po
1.0
0.8
0.6
0.4
0.2
0.0
10 nM
100 nM
1 μM
10 μM
100 μM
[Ca2+]i
[IP3] Kinh
10μM 63μM
180 μM —
100 nM —
Pmax = 0.81
Kact ~ 210nM
Hact ~ 1.8
Hinh ~ 3.9


In the presence of sub-saturating [InsP3]
[InsP3] = 33 nM
[Ca2+]i
250 nM
7 pA
100 ms
1.1 μM
21 μM
Oocyte nuclei isolated directly into bath solution with [Ca2+] ≈ 450 nM

Ca^{2+} response of $InsP_3R$ channel in various $[InsP_3]$

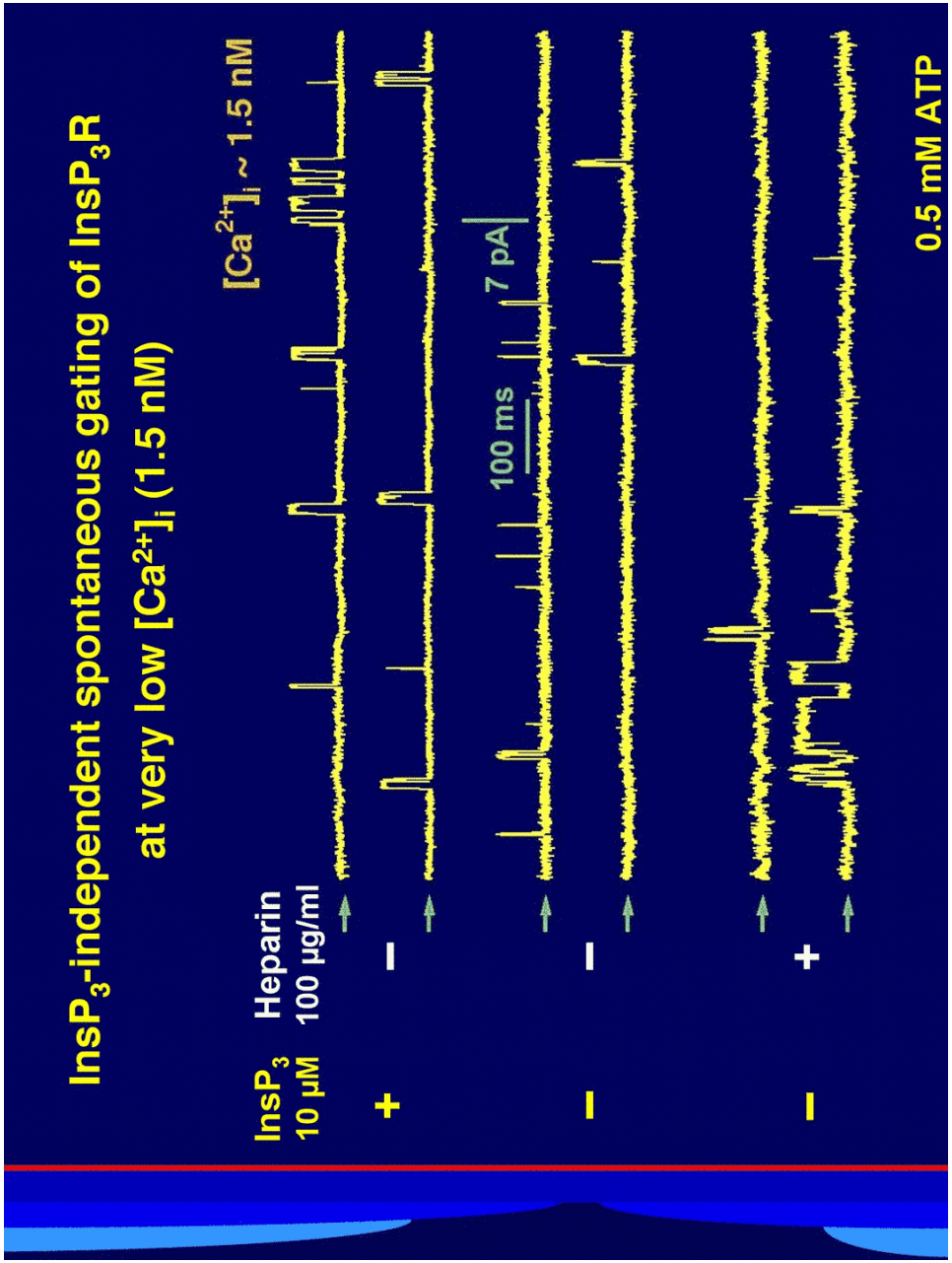


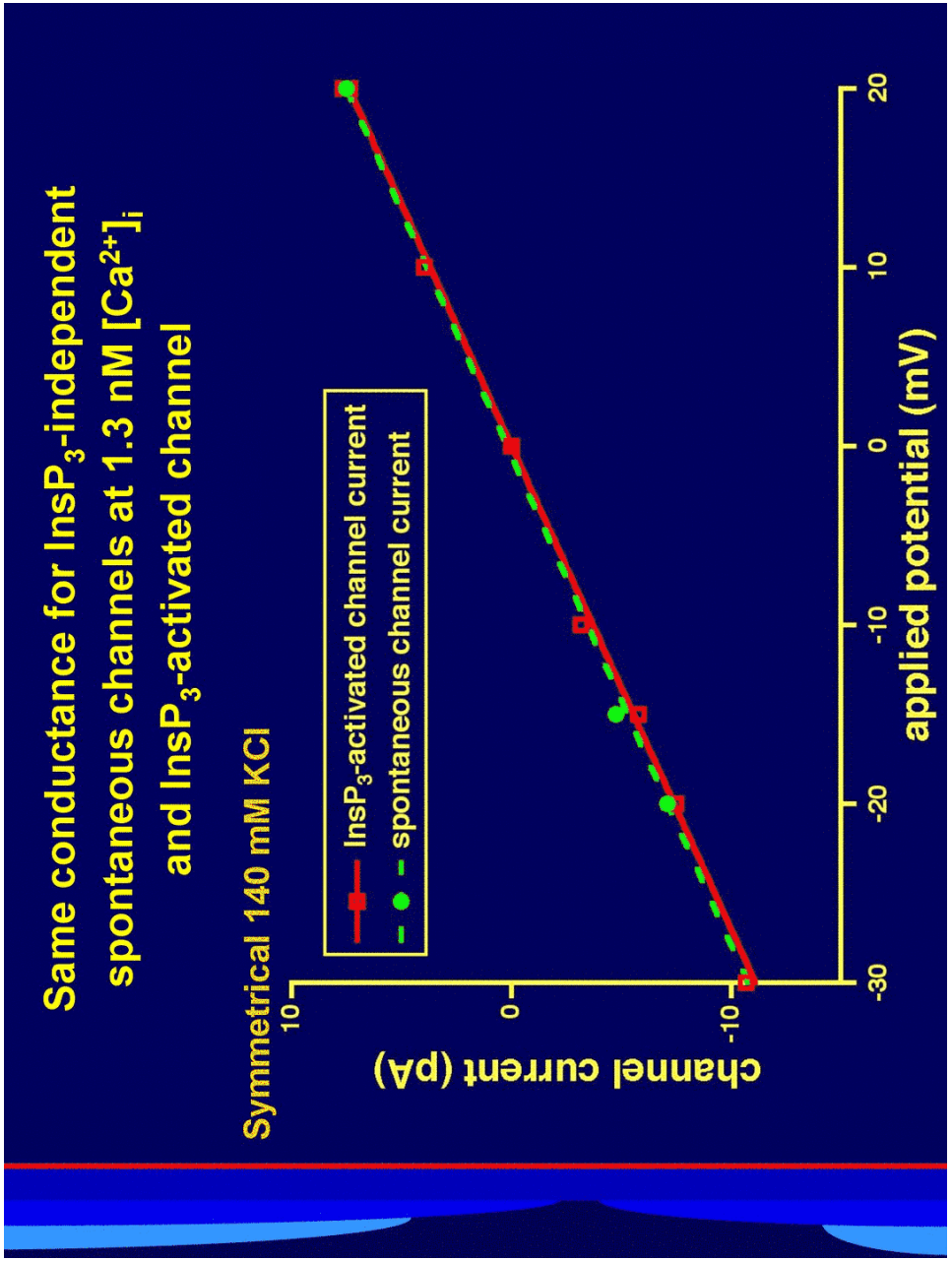
- **Experimental observations:**

  (A) Regulation of $InsP_3R$ channel activities by $[Ca^{2+}]_i$

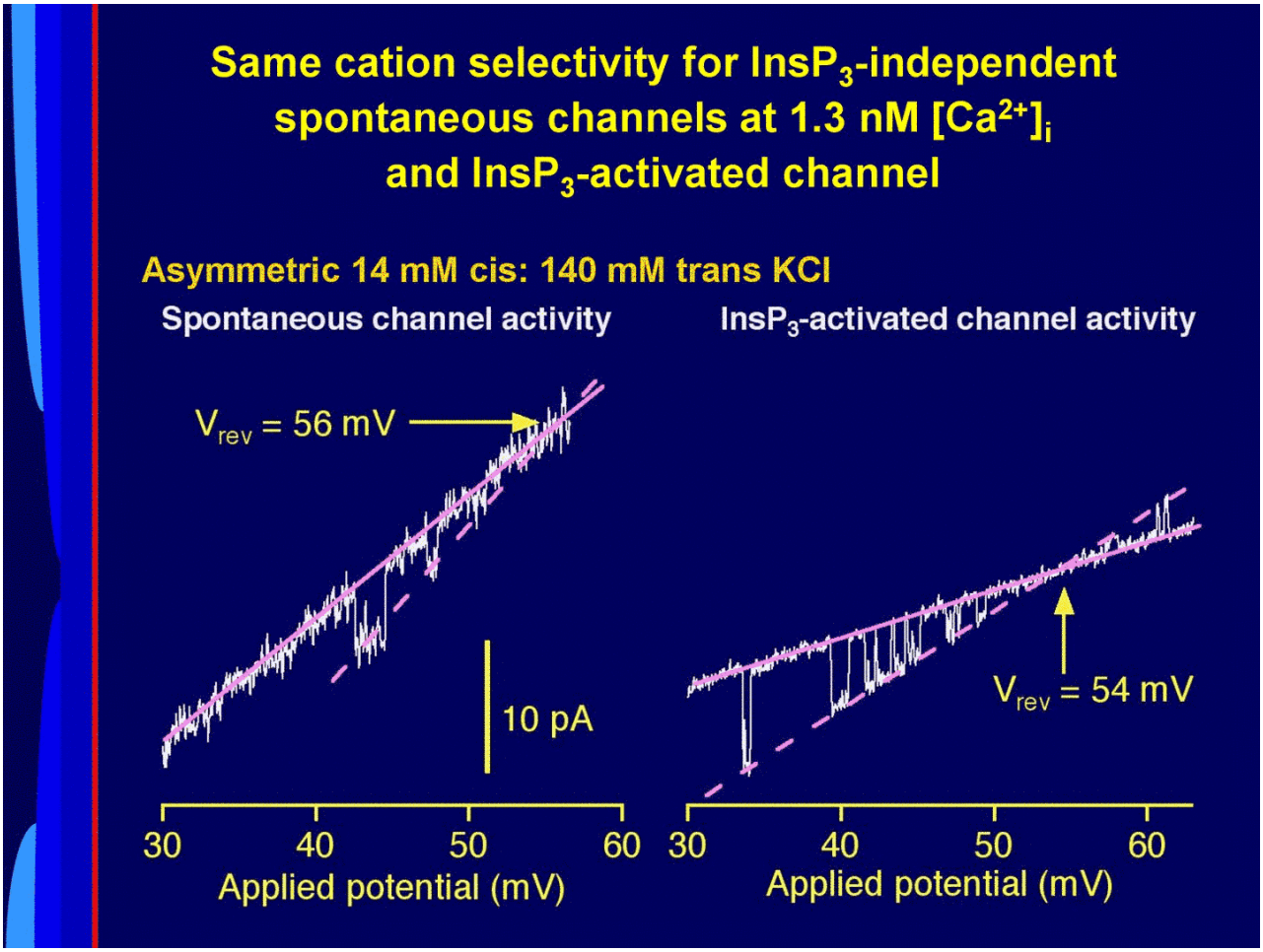
  (B) Effects of $[InsP_3]$ on $InsP_3R$ channel activities

  (C) Spontaneous $InsP_3R$ channel activity in the absence of ligand

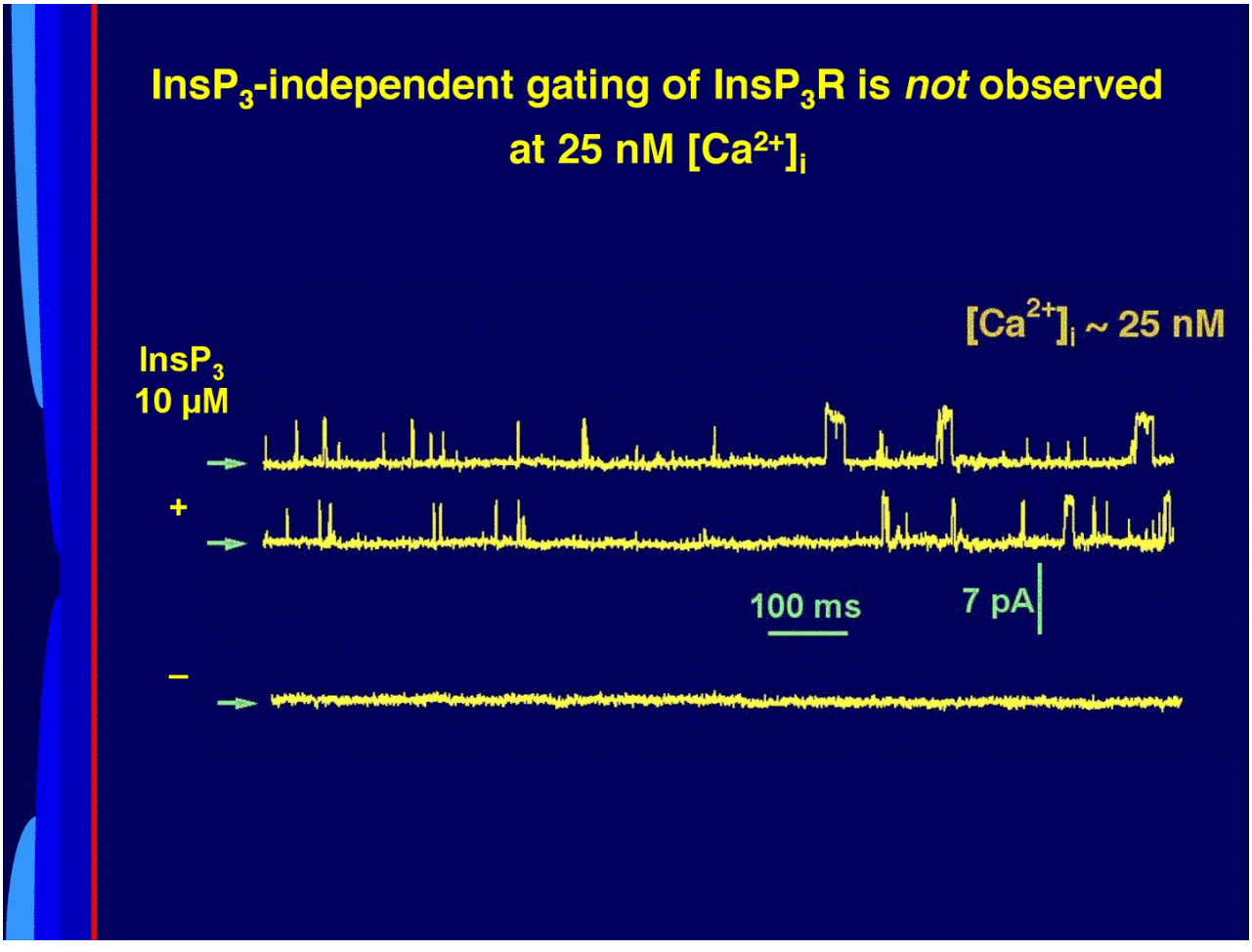

InsP3-independent spontaneous gating of InsP3R
at very low [Ca2+]i (1.5 nM)
[Ca2+]i ~ 1.5 nM
InsP3
10 µM
Heparin
100 µg/ml
100 ms
7 pA
0.5 mM ATP


Same conductance for InsP3-independent
spontaneous channels at 1.3 nM [Ca2+]i
and InsP3-activated channel
Symmetrical 140 mM KCl
InsP3-activated channel current
spontaneous channel current
channel current (pA)
applied potential (mV)

Same cation selectivity for $InsP_3$-independent spontaneous channels at 1.3 nM $[Ca^{2+}]_i$ and $InsP_3$-activated channel



$InsP_3$-independent gating of $InsP_3R$ is *not* observed at 25 nM $[Ca^{2+}]_i$



- **Experimental observations:**

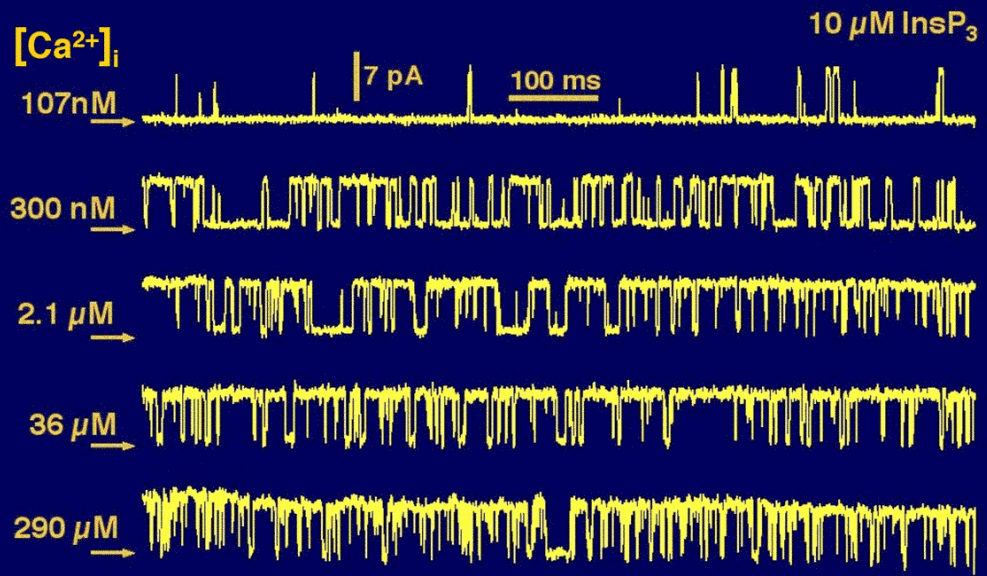
  (A) Regulation of $InsP_3R$ channel activities by $[Ca^{2+}]_i$

  (B) Effects of $[InsP_3]$ on $InsP_3R$ channel activities

  (C) Spontaneous $InsP_3R$ channel activity in the absence of ligand

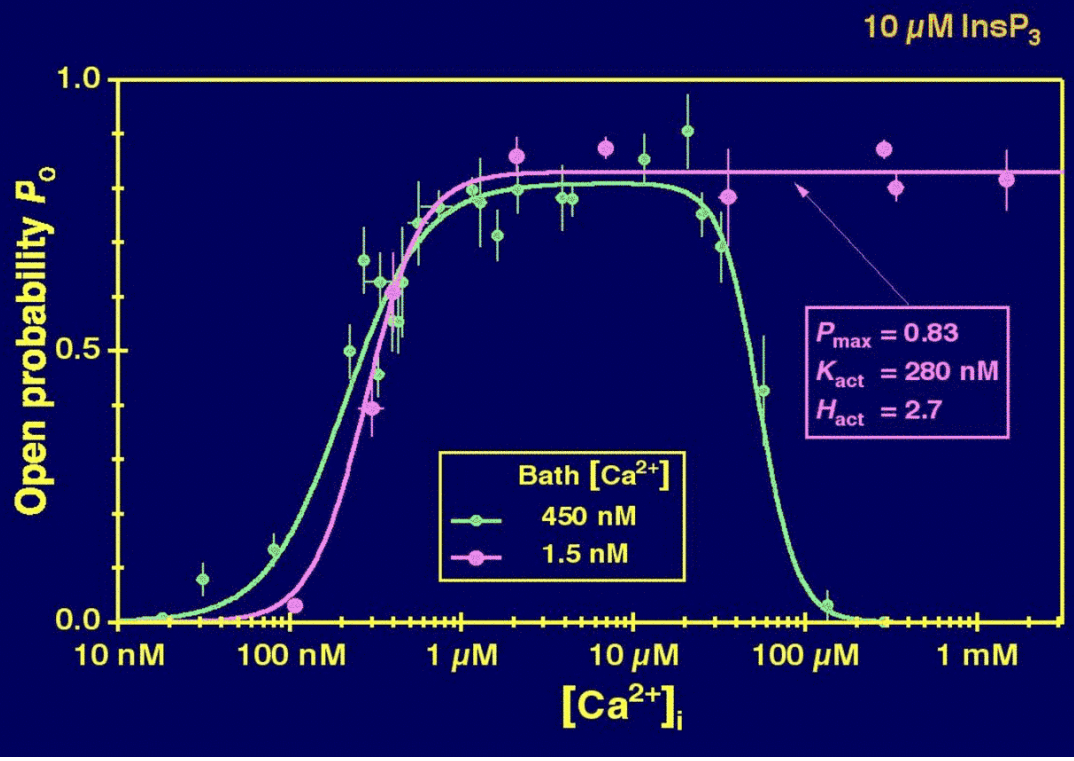
  (D) Channel activities of $InsP_3R$ exposed to ultra-low $[Ca^{2+}]$

$InsP_3R$ channel gating after exposure of nuclei to ultra-low bath $[Ca^{2+}]$ (1.5 nM)

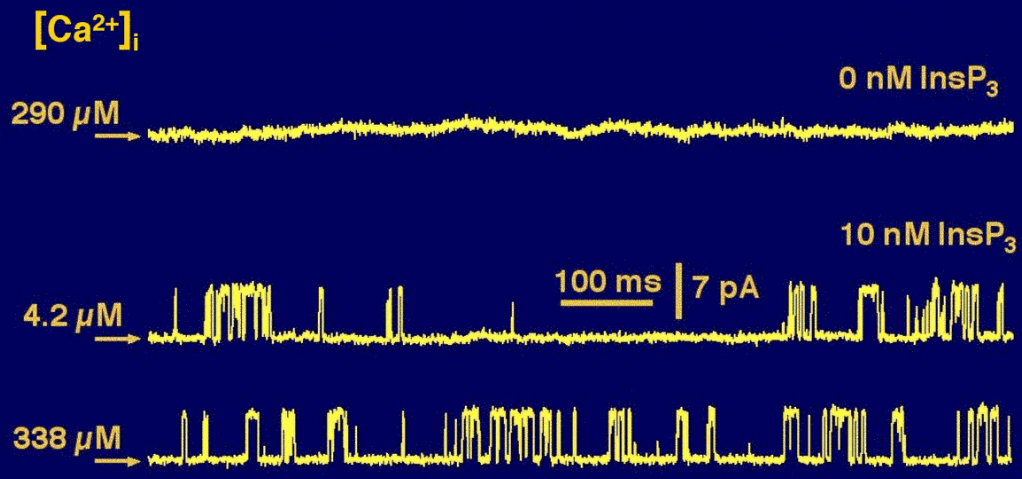


Oocyte nuclei isolated into bath solution with $[Ca^{2+}] \sim 1.5$ nM and incubated for 20 min.

Lack of high-$[Ca^{2+}]_i$ inhibition of $InsP_3R$ channel gating after exposure to ultra-low bath $[Ca^{2+}]$ (1.5 nM)

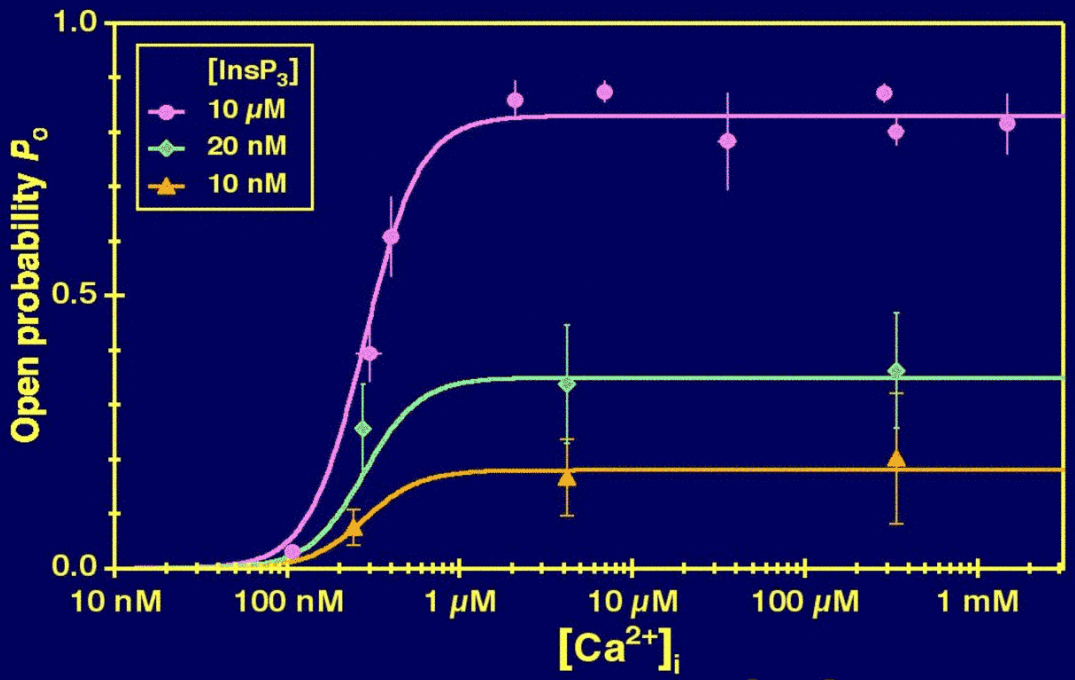


$InsP_3R$ channel gating is still $InsP_3$ dependent despite lack of high-$[Ca^{2+}]_i$ inhibition after exposure to ultra-low $[Ca^{2+}]$ bath



Oocyte nuclei isolated into bath solution with $[Ca^{2+}] \sim 1.5$ nM and incubated for 20 min.

Ca^{2+} response in various $[InsP_3]$ for $InsP_3R$ channels exposed to ultra-low $[Ca^{2+}]$ bath



Oocyte nuclei isolated into bath solution with $[Ca^{2+}] \sim 1.5$ nM and incubated for 20 min.

The maximum P_o for $InsP_3R$ channel is 0.8 (< 1) even under optimal $[InsP_3]$ and $[Ca^{2+}]_i$ with or without high-$[Ca^{2+}]_i$ inhibition

$[InsP_3] = 10\ \mu M$

$[Ca^{2+}]_i$

Oocyte nuclei isolated directly into bath solution with $[Ca^{2+}] \approx 450$ nM

Even when inhibition by high $[Ca^{2+}]_i$ is relieved:

Oocyte nuclei isolated into bath solution with $[Ca^{2+}] \sim 1.5$ nM and incubated for 20 min.

Modeling for $InsP_3R$ channel gating

Modeling for $InsP_3R$ channel gating

Model must account for the functional observations:

» **For nuclei isolated into bath solution with 450 nM Ca^{2+}:**

> the biphasic regulation of $InsP_3R$ channel gating by $[Ca^{2+}]_i$

> $InsP_3$ regulation of $InsP_3R$ gating mostly affects the half-maximal inhibitory $[Ca^{2+}]_i$ and does not affects Ca^{2+} activation parameters

> High sensitivity of $InsP_3R$ to $[InsP_3]$ over a narrow range (< 100 nM)

> Abrupt saturation of $[InsP_3]$ regulation beyond 100 nM

> Spontaneous gating of $InsP_3R$ when all its ligand binding sites (Ca^{2+} or $InsP_3$) are unoccupied

Modeling for $InsP_3R$ channel gating

Model must account for the functional observations:

» For nuclei isolated into bath solution with 1.5 nM Ca^{2+}:

> Activation of $InsP_3R$ channel gating at low $[Ca^{2+}]_i$ (0.1 – 1 μM) is similar to that observed in 450 nM bath $[Ca^{2+}]$

> Absence of inhibition of channel gating at high $[Ca^{2+}]_i$

> Gating of $InsP_3R$ remains $InsP_3$ dependent

> In sub-saturating $[InsP_3]$, $[InsP_3]$ mostly affects the maximum channel P_o the channel exhibits

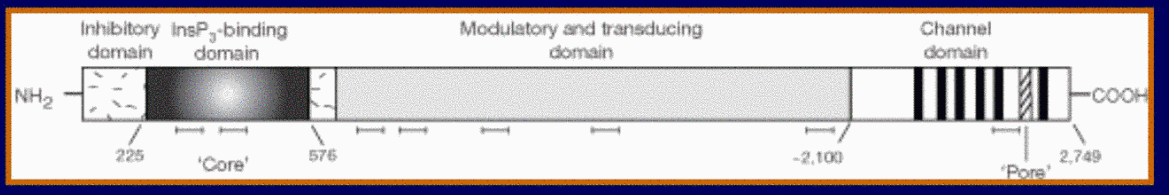
> Even with the full abrogation of high $[Ca^{2+}]_i$ inhibition, maximum channel P_o observed is 0.8, < 1

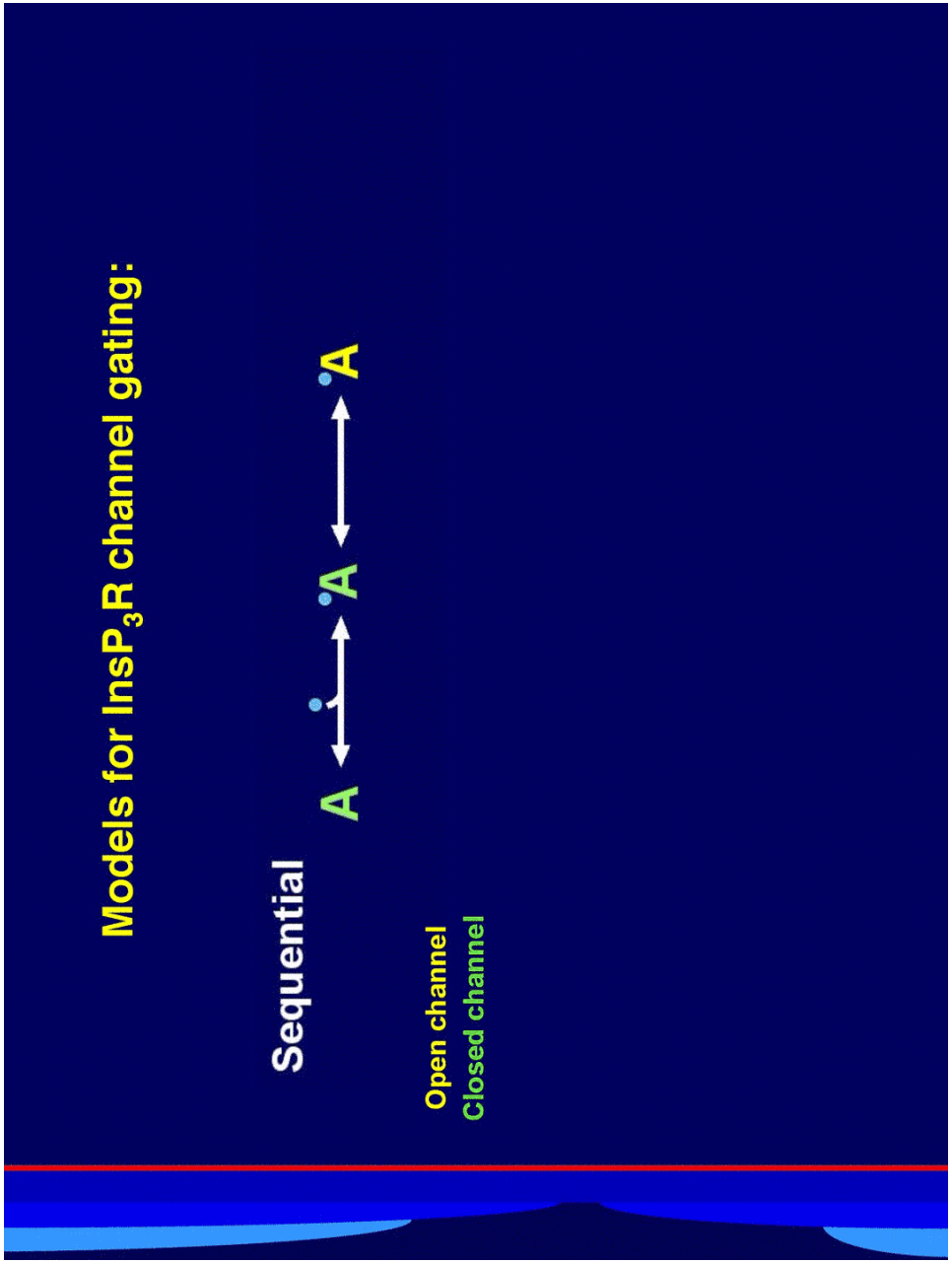
Modeling for $InsP_3R$ channel gating

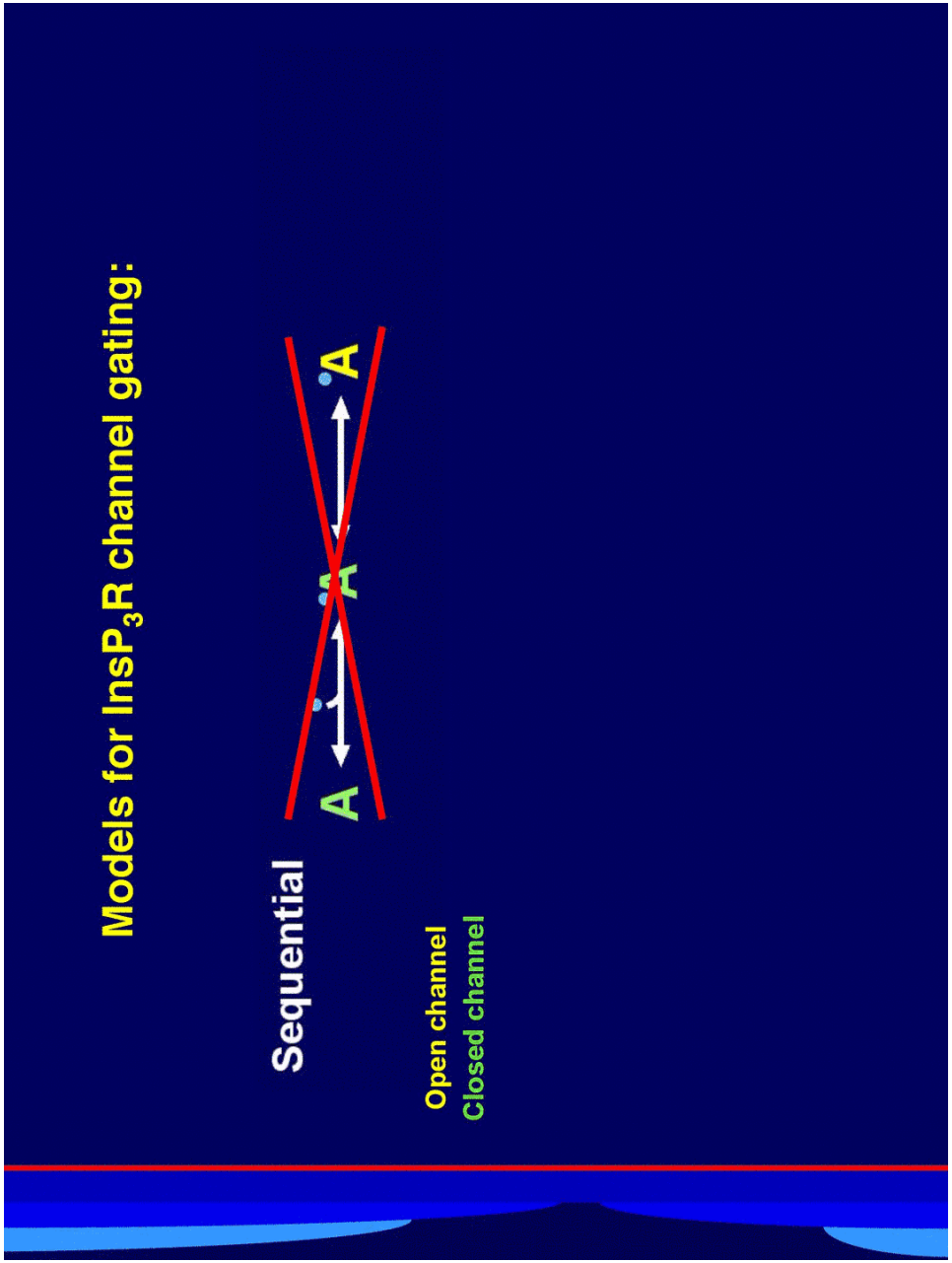
Model must also take into account the molecular structure of $InsP_3R$ channel

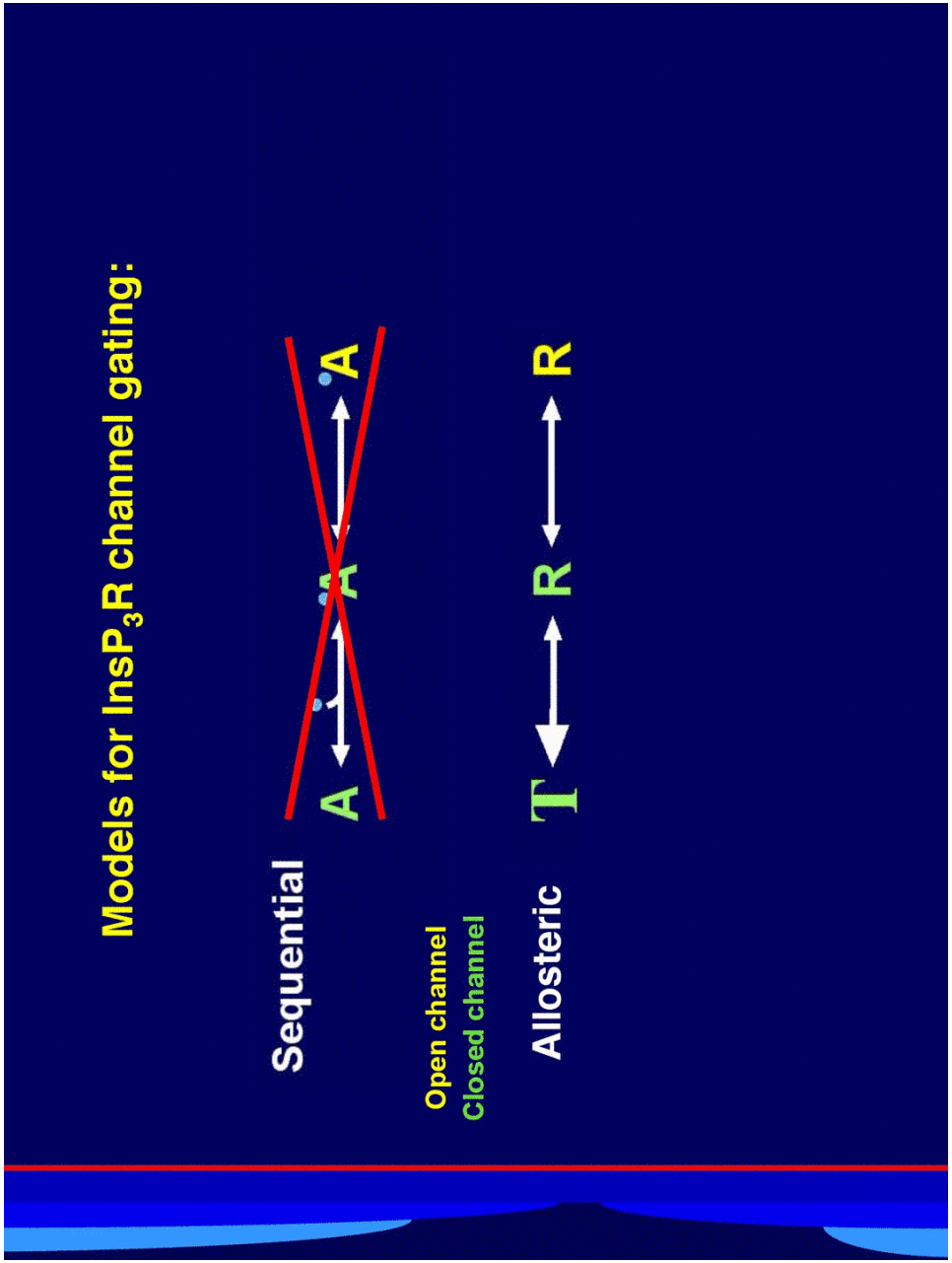
» Functional $InsP_3R$ channel is a tetramer of four $InsP_3R$ molecules

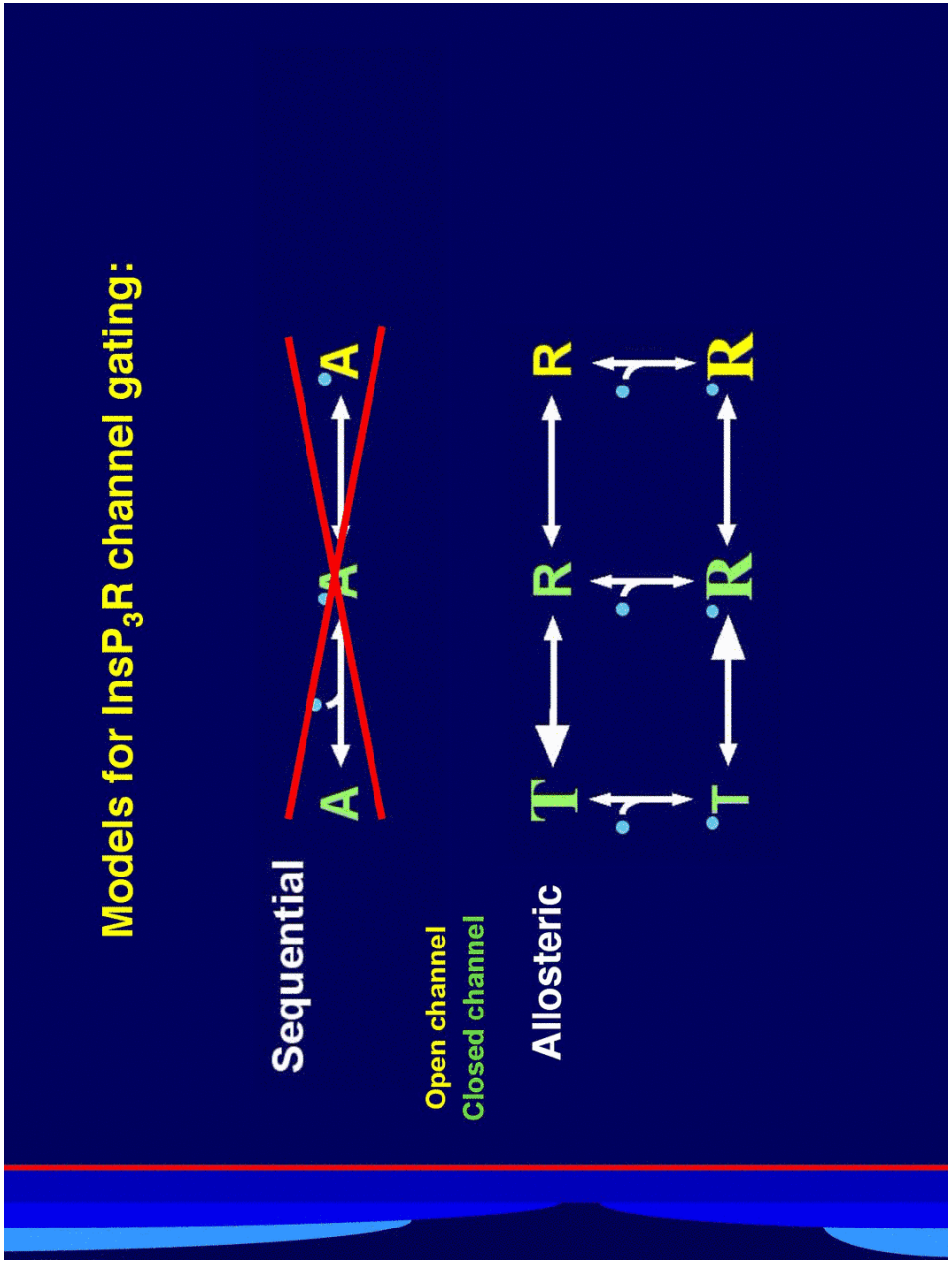
» While each $InsP_3R$ molecule has multiple potentially functional Ca^{2+} binding sites, only one $InsP_3$ binding site has been identified.




Models for InsP3R channel gating:
Sequential
A
A
A
Open channel
Closed channel


Models for InsP3R channel gating:
Sequential
A
A
A
Open channel
Closed channel


Models for InsP3R channel gating:
Sequential
A
A
A
Open channel
Closed channel
Allosteric
T
R
R

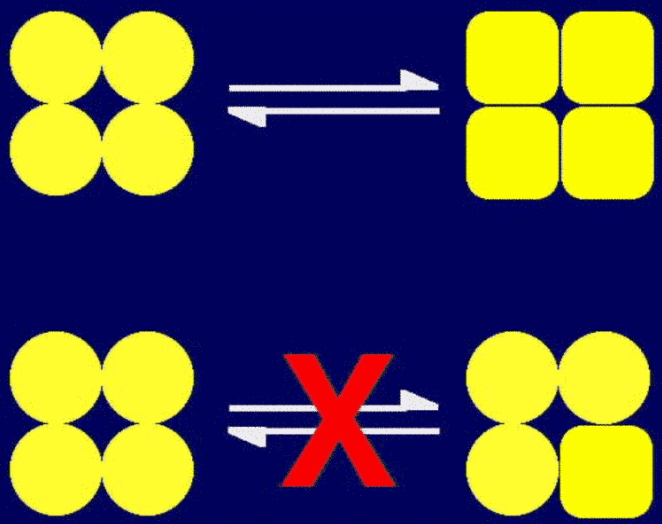

Models for InsP3R channel gating:
Sequential
A
A
A
Open channel
Closed channel
Allosteric
T
R
R
T
R
R

Simplest model for $InsP_3R$ channel gating (one that involves the fewest free parameters)

Allosteric model based on Monod-Wyman-Changeux (MWC) model

Parameter-reducing simplifications assumed in MWC allosteric model

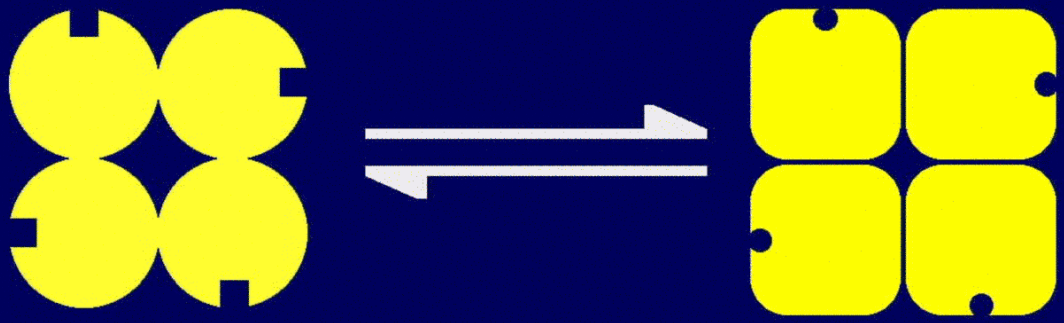
» MWC model assumes that the four monomers in the channel always adopt the same conformation, changing between two conformations concertedly.



Parameter-reducing simplifications assumed in MWC allosteric model

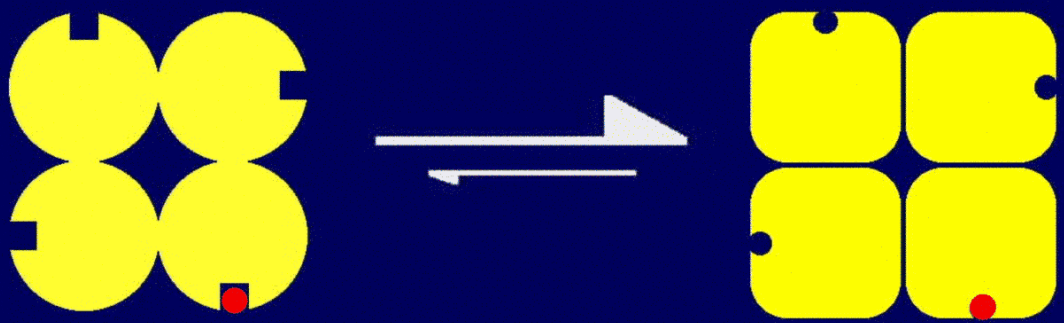
» The equivalent ligand binding sites of all the monomers in one tetrameric $InsP_3R$ channel have the same affinity at any time.

» In different conformations of the channel, ligand binding sites can exhibit different affinities.



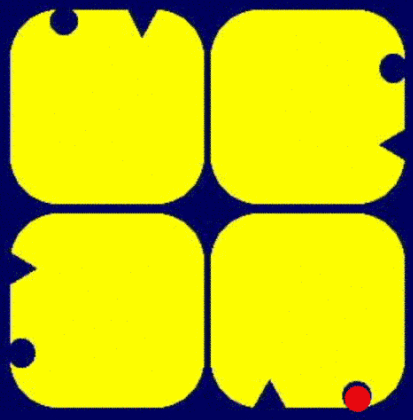
Parameter-reducing simplifications assumed in MWC allosteric model

» Ligand binding to the channel stabilizes the conformation in which the binding sites have higher affinity.

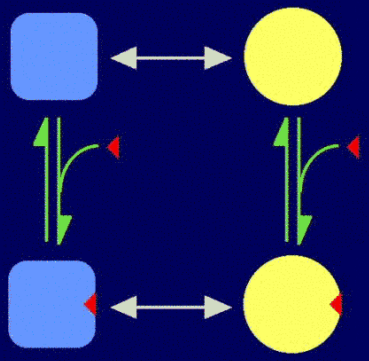


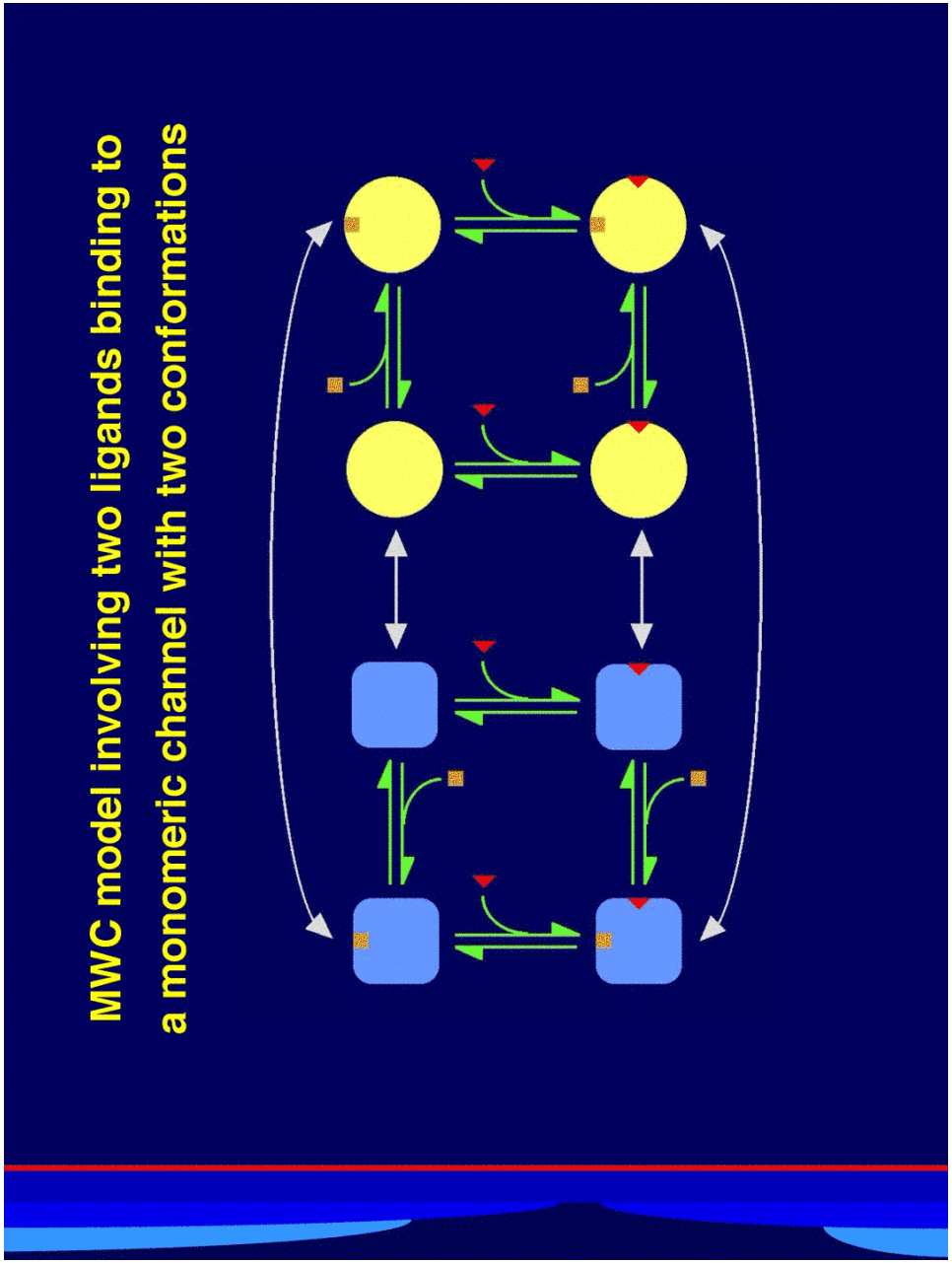
Parameter-reducing simplifications assumed in MWC allosteric model

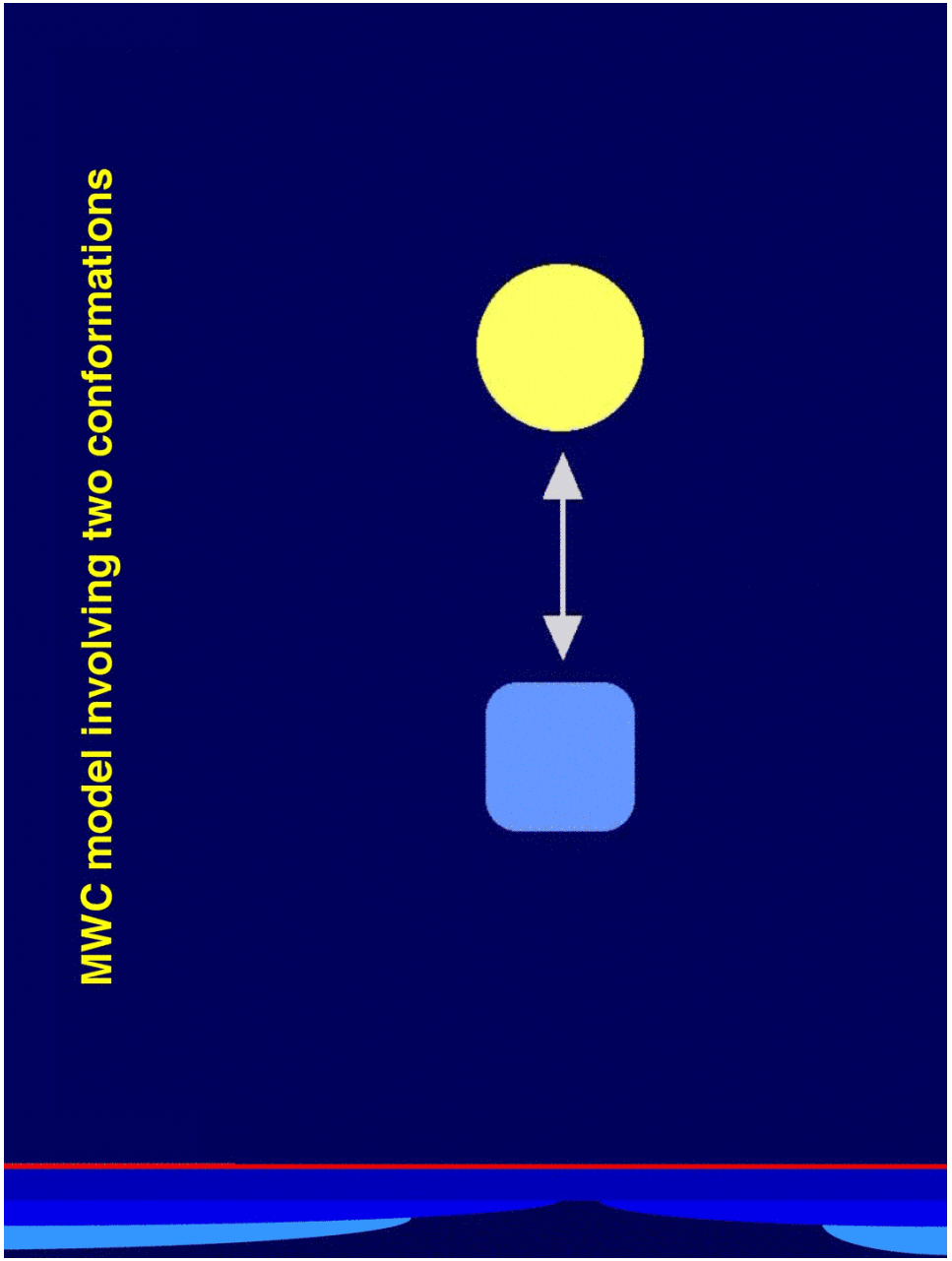
» The affinity of any ligand binding site in a channel is *not* affected by the state of occupancy of the other ligand binding sites in that channel.



MWC model involving one ligand binding to a monomeric channel with two conformations




MWC model involving two ligands binding to
a monomeric channel with two conformations


MWC model involving two conformations

How many functional Ca^{2+} binding sites are there?

» One activating binding site (F) for activation of the channel at 200 nM $[Ca^{2+}]_i$.

> This site is $InsP_3$ independent.

» One inhibitory binding site (H) for high-$[Ca^{2+}]_i$ inhibition of channel gating.

> This site becomes non-functional after exposure of the channel to ultra-low $[Ca^{2+}]$ bath to relieve high-$[Ca^{2+}]_i$ inhibition

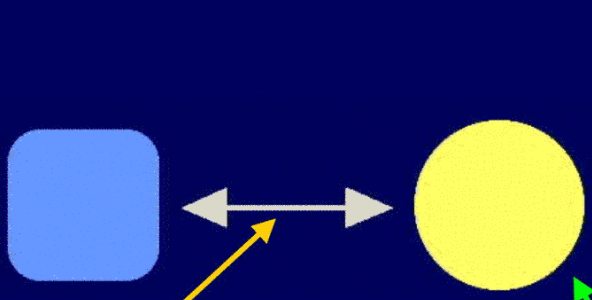
» One binding site (G) must be $InsP_3$ dependent and inhibitory in the absence of $InsP_3$.

> This site keeps the channel inactive in the absence of $InsP_3$ even when the H site becomes non-functional after exposure of the channel to ultra-low $[Ca^{2+}]$ bath

Each $InsP_3R$ monomer has three functional Ca^{2+} binding sites on the cytoplasmic side of the channel

MWC model involving two conformations

In a tetrameric $InsP_3R$ channel, there are 5 $InsP_3$-binding states. Similarly, there are 5 binding states for each of three Ca^{2+}-binding sites. Total of $5 \times 5^3 = 625$ different ligand-binding states for this conformation



625 conformation changes, each between 2 conformations in the same ligand-binding state

625 different ligand-binding states for this conformation

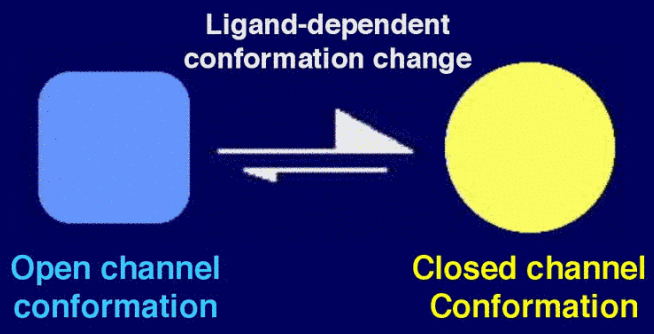
Simplest model for $InsP_3R$ channel gating:

- The $InsP_3R$ channel can assume six functionally distinct conformations

  Why six?

Two-conformation MWC model:

In the absence of ligand



Two-conformation MWC model:

Any ligand that stabilizes the open conformation can activate the channel independent of any other ligands

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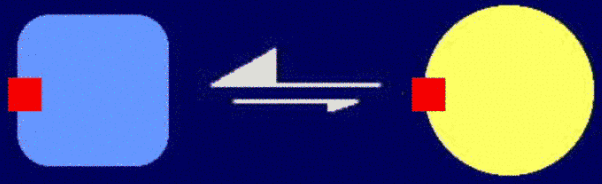
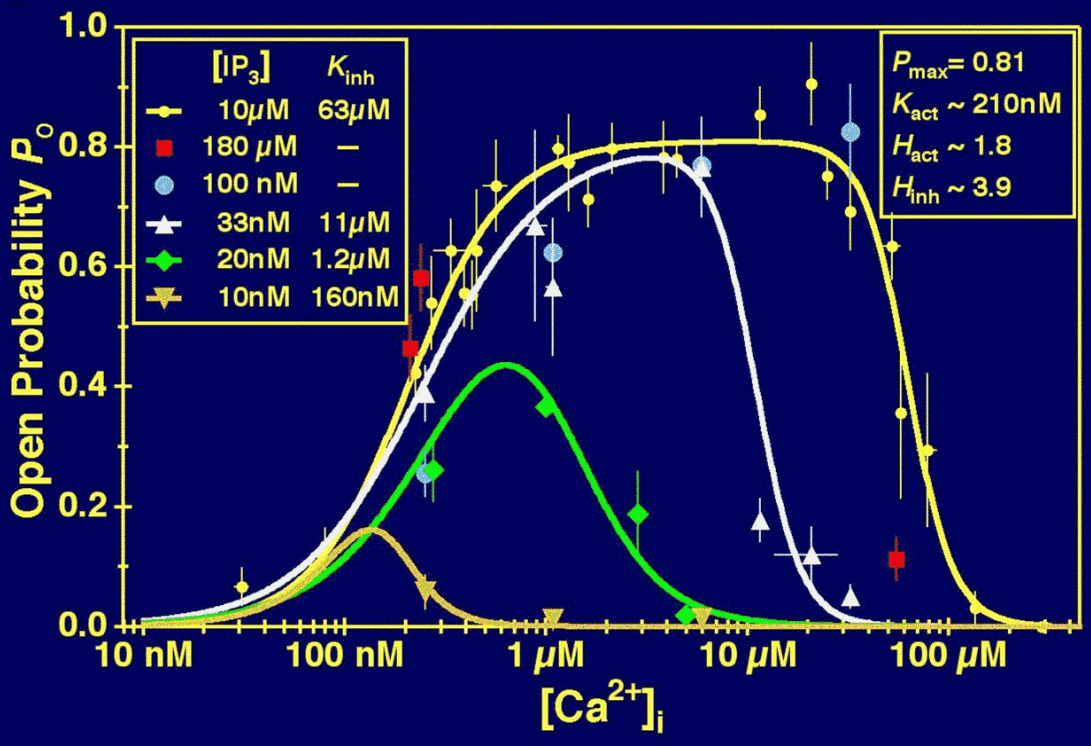
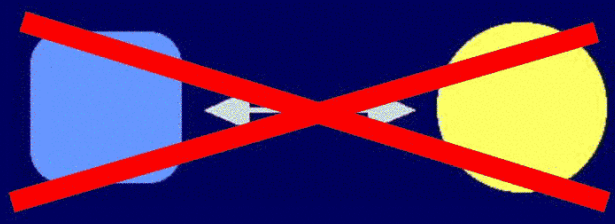


Ca^{2+} response of $InsP_3R$ channel in various $[InsP_3]$



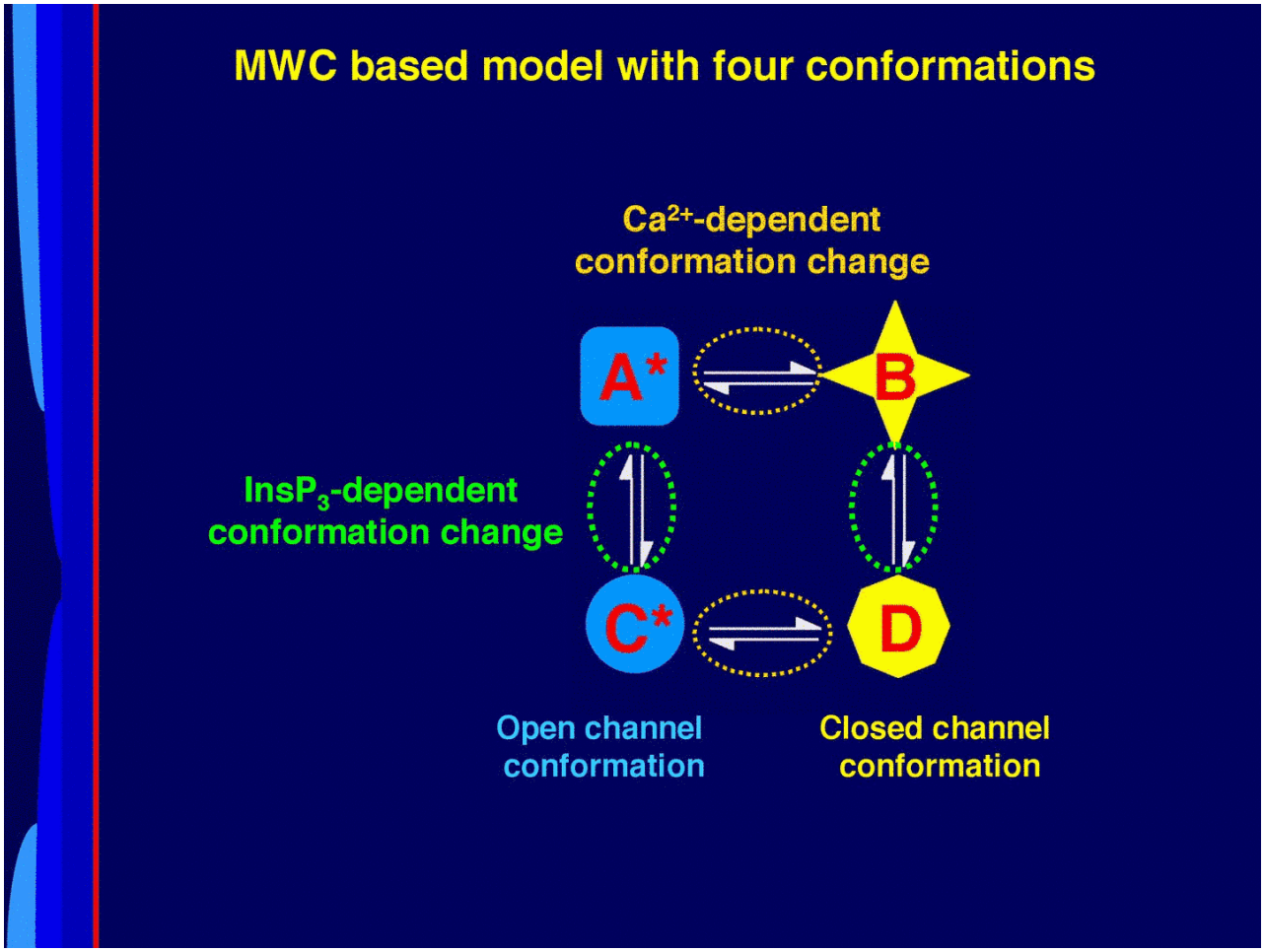
Two-conformation MWC model:

Any ligand that stabilizes the open conformation can activate the channel independent of any other ligands

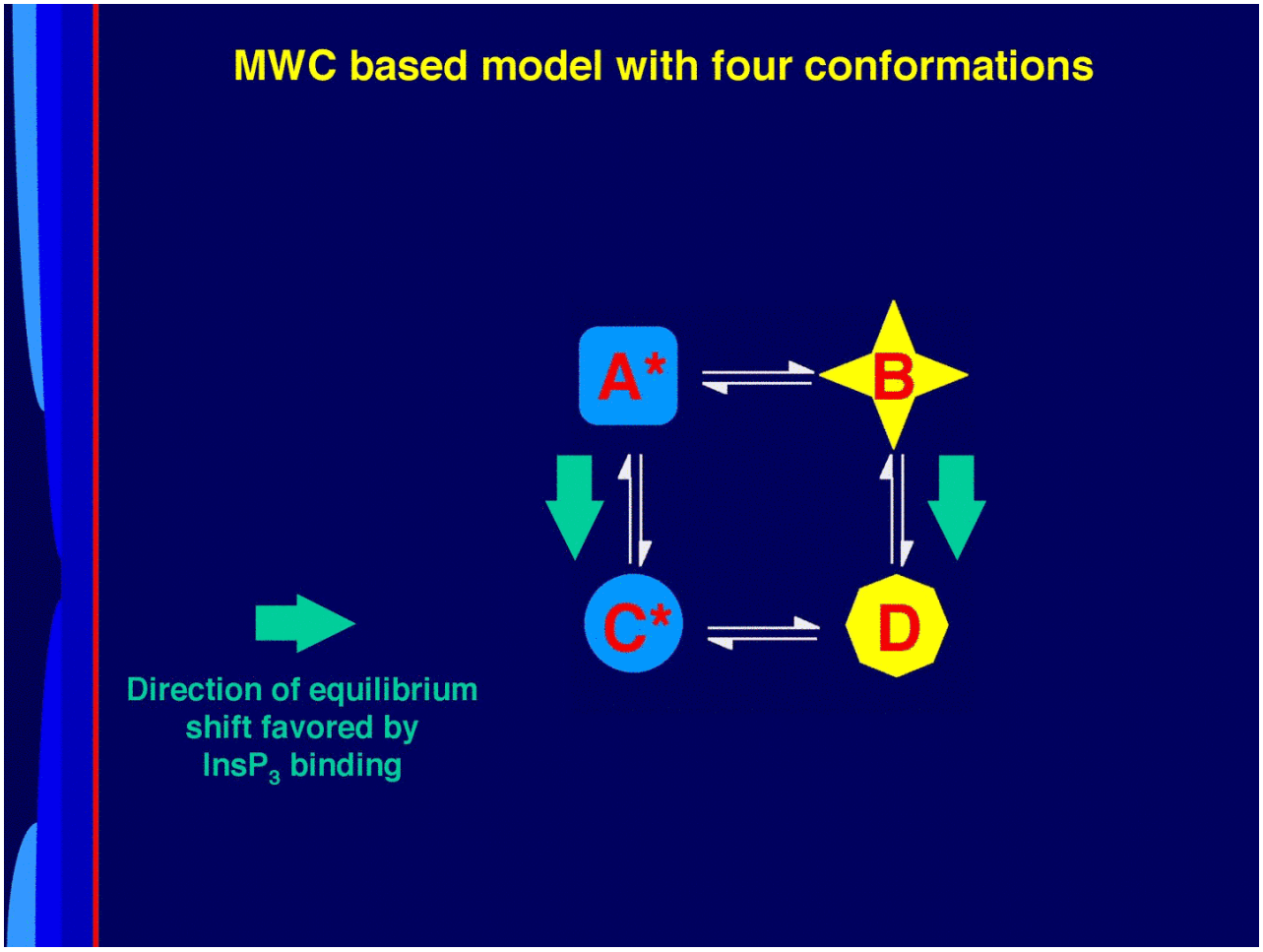


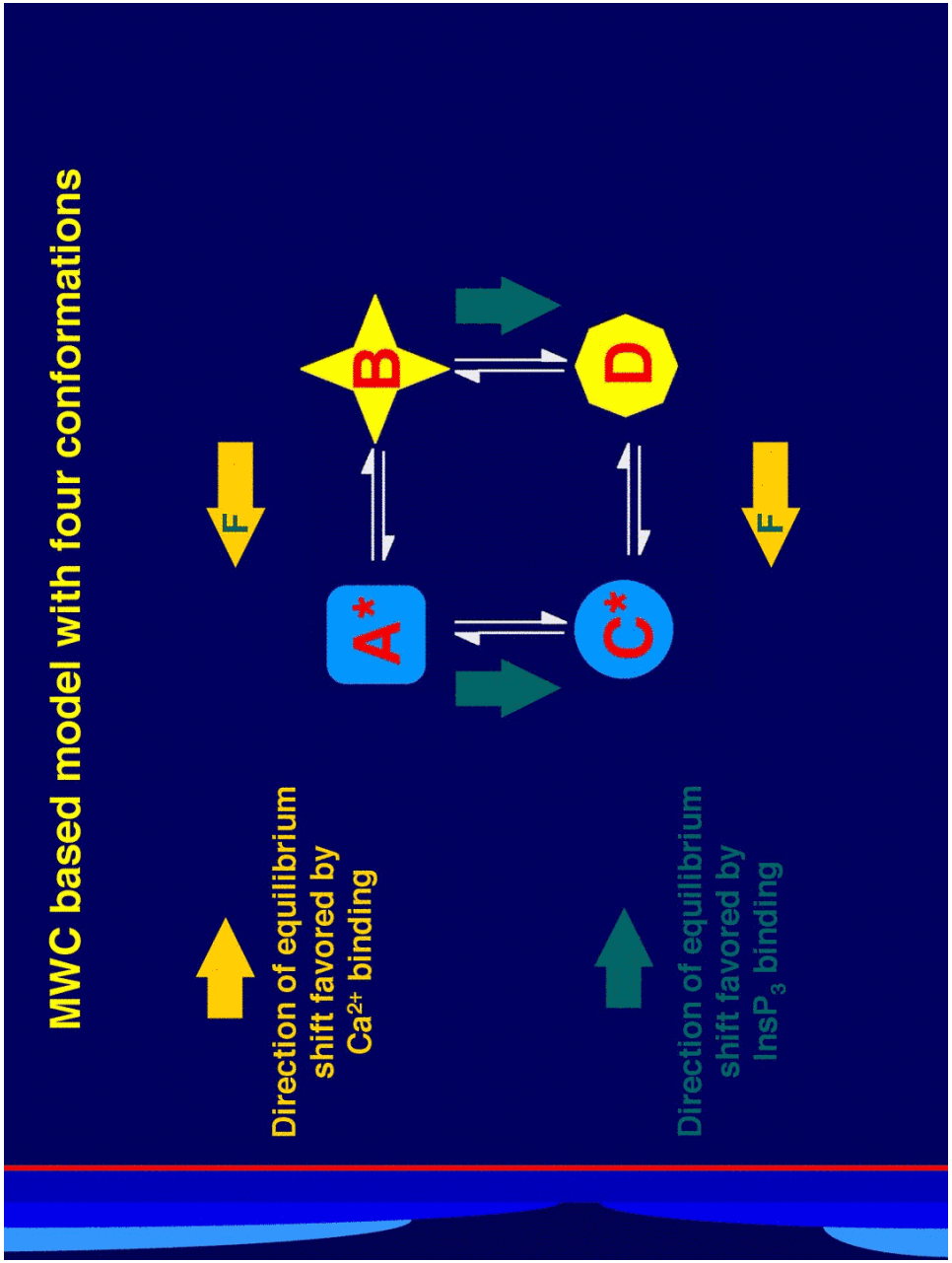
Neither $InsP_3$ nor Ca^{2+} alone can activate the channel

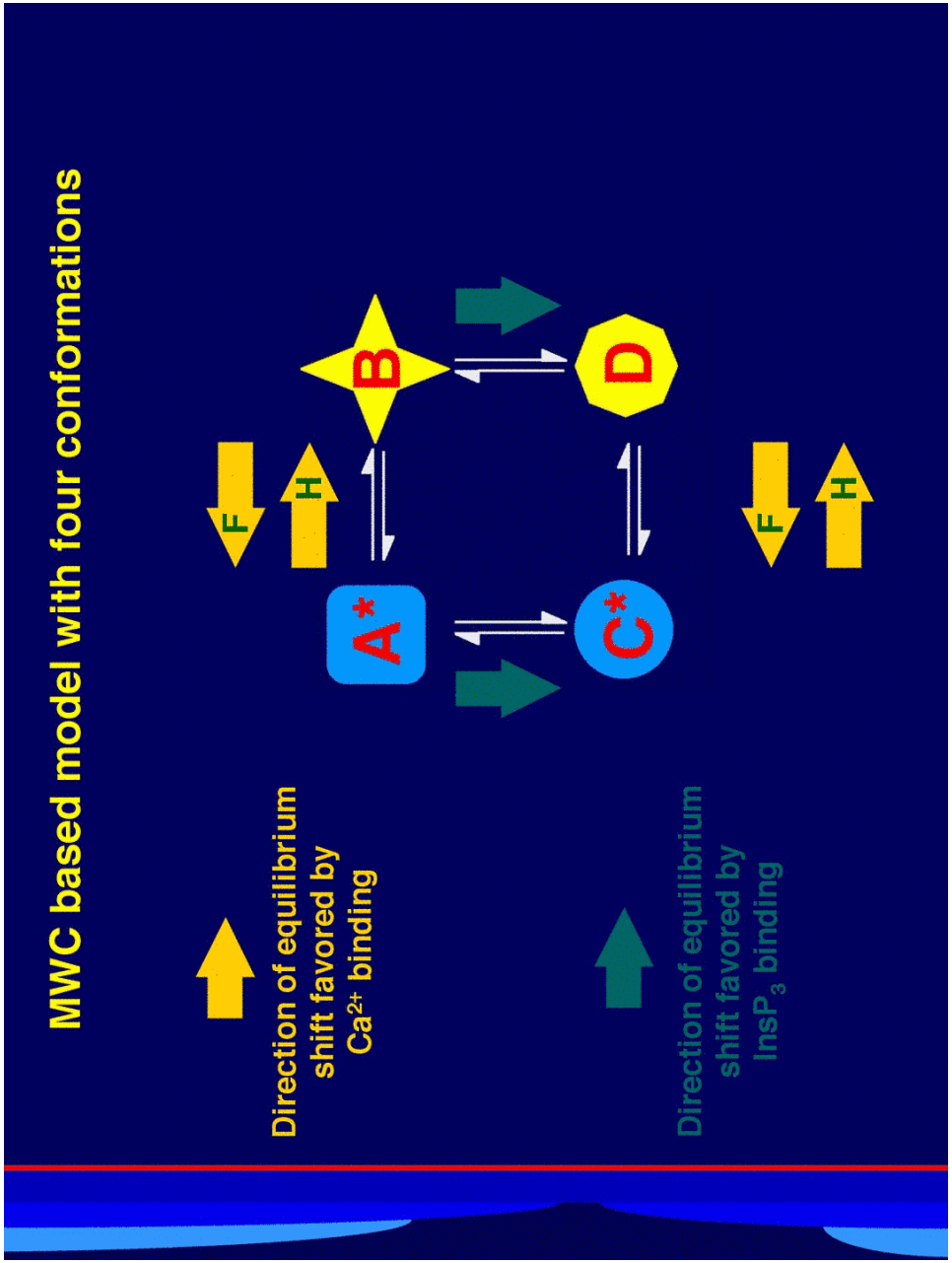
MWC based model with four conformations



MWC based model with four conformations



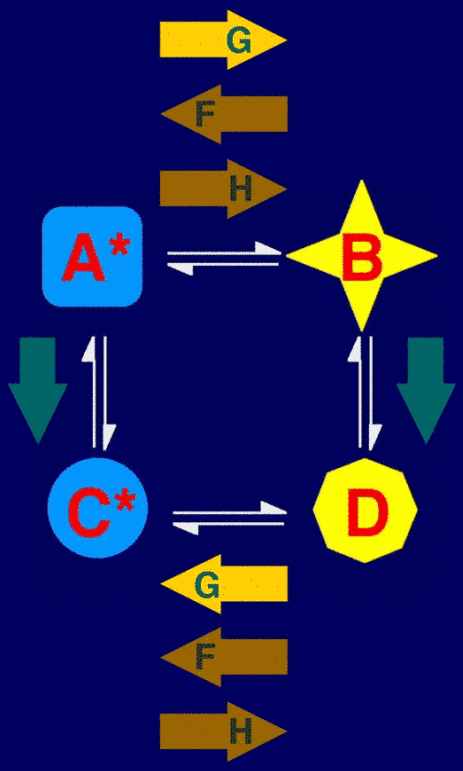

MWC based model with four conformations
B
D
A*
C*
F
F
Direction of equilibrium shift favored by Ca^{2+} binding
Direction of equilibrium shift favored by $InsP_3$ binding


MWC based model with four conformations
B
D
A*
C*
F
H
F
H
Direction of equilibrium shift favored by Ca^{2+} binding
Direction of equilibrium shift favored by $InsP_3$ binding

MWC based model with four conformations

Direction of equilibrium shift favored by Ca^{2+} binding

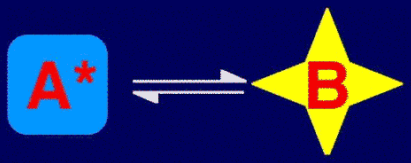
Direction of equilibrium shift favored by $InsP_3$ binding



MWC based model with four conformations

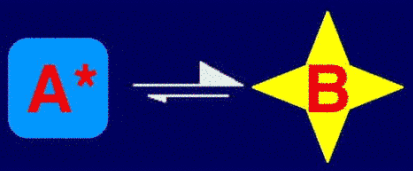
In the absence of $InsP_3$,

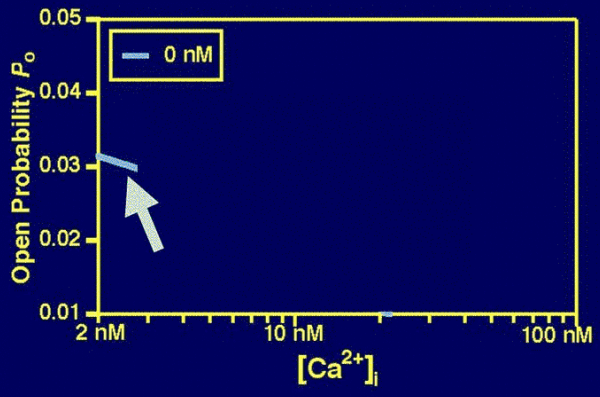
conformations A* and B are more favorable than conformations C* and D



MWC based model with four conformations

In the absence of $InsP_3$





In 1.5 nM $[Ca^{2+}]_i$, none of the Ca^{2+} binding sites is occupied

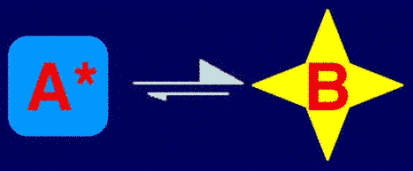
B is the dominant conformation

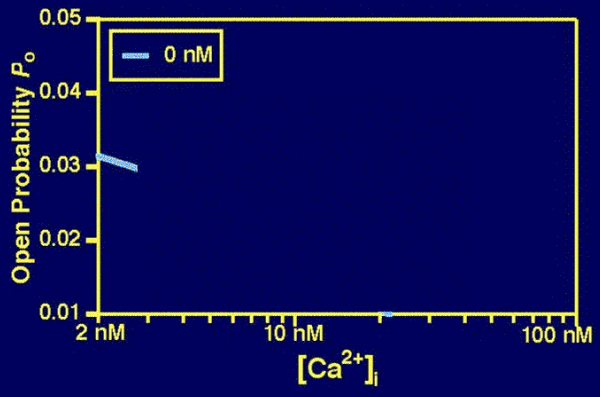
but channel has observable probability to enter A* conformation

➔ spontaneous gating

MWC based model with four conformations

In the absence of $InsP_3$





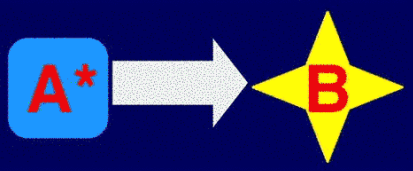
Among the 3 Ca^{2+} binding sites in both conformations,

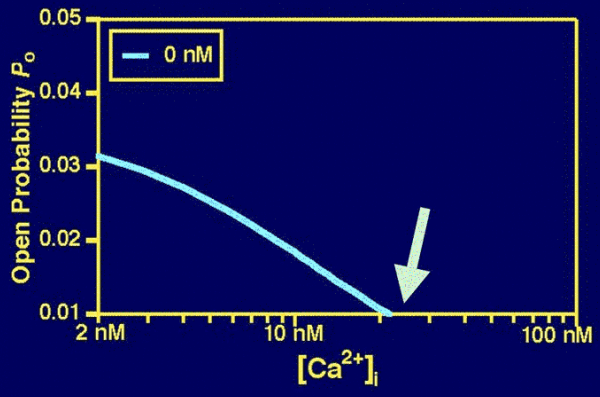
G site in B conformation has the highest affinity.

So the G site is inhibitory

MWC based model with four conformations

In the absence of $InsP_3$





As $[Ca^{2+}]_i$ increases (from 1.5 nM to 25 nM),

Ca^{2+} bind to G sites, further stabilizing closed B conformation

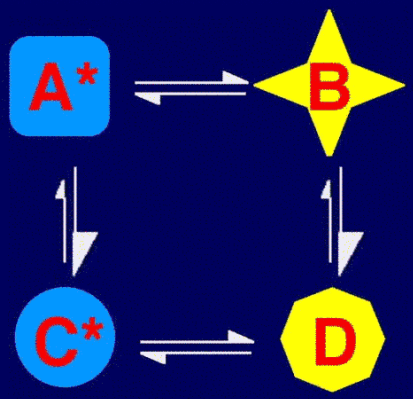
➔ Channel gating is inhibited by $[Ca^{2+}]_i$ = 25 nM

F site in A* conformation has the next highest affinity.

So the F site is activating. But it is insufficient to counter the Inhibition of the channel by the G site.

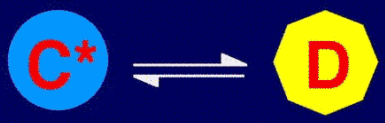
MWC based model with four conformations

$InsP_3$ binding to channel stabilizes conformations C* and D relative to conformations A* and B



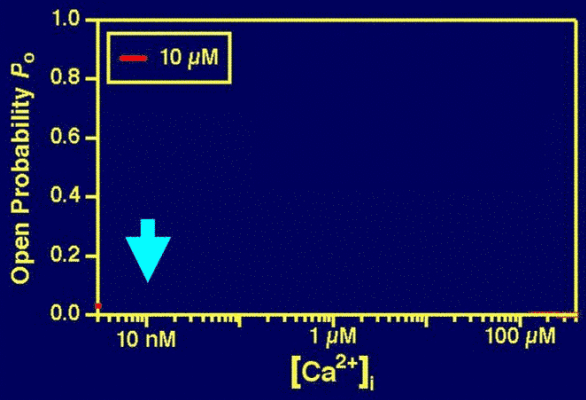
MWC based model with four conformations

In saturating [$InsP_3$]



MWC based model with four conformations

In saturating [$InsP_3$]



In low $[Ca^{2+}]_i$, when none of the Ca^{2+} binding sites is occupied,

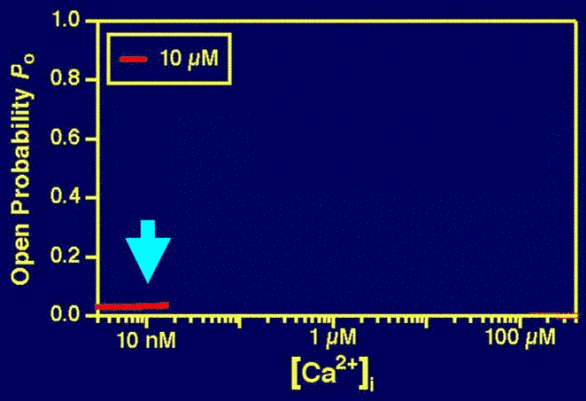
D is the dominant conformation.

Channel has observable probability to enter C* conformation

➔ low but detectable channel P_o

MWC based model with four conformations

In saturating [$InsP_3$]



In low $[Ca^{2+}]_i$, when none of the Ca^{2+} binding sites is occupied,

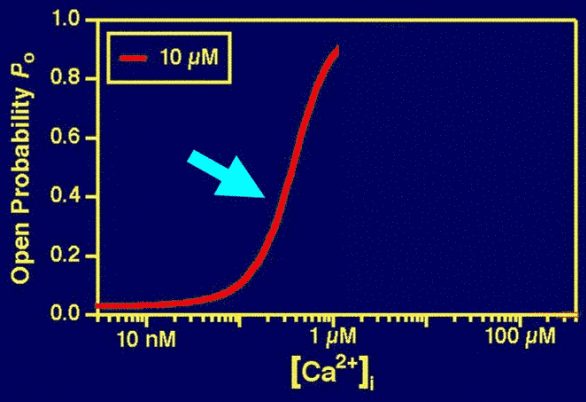
D is the dominant conformation.

Channel has observable probability to enter C* conformation

➔ low but detectable channel P_o

MWC based model with four conformations

In saturating [$InsP_3$]



Among the 3 Ca^{2+} binding sites in both conformations,

G site in C* conformation has the highest affinity.

F site in C* conformation has the next highest affinity.

So both G and F sites are now activating

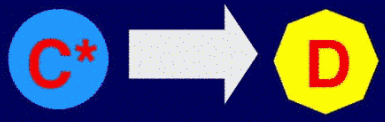
As $[Ca^{2+}]_i$ increases (from 1.5 to 1000 nM),

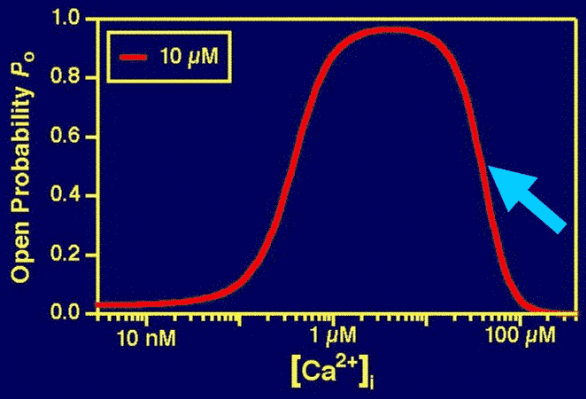
Ca^{2+} bind to G and then F sites, stabilizing open C* conformation

➔ Ca^{2+} activation of $InsP_3R$ channel gating

MWC based model with four conformations

In saturating [$InsP_3$]





As $[Ca^{2+}]_i$ further increases to > 20 μM,

Ca^{2+} then bind to H site in D conformation,

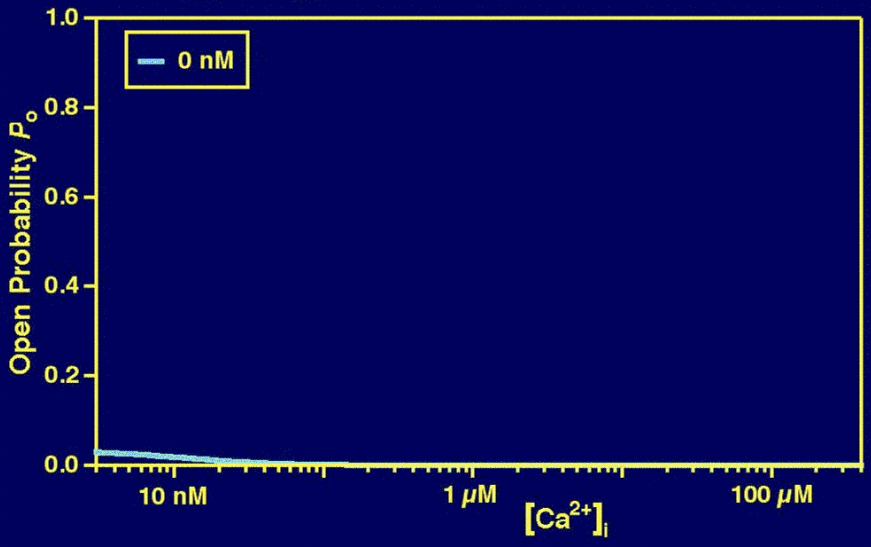
stabilizing closed D conformation

➔ Ca^{2+} inhibition of $InsP_3R$ channel gating

➔ Biphasic Ca^{2+} regulation of $InsP_3R$ channel gating

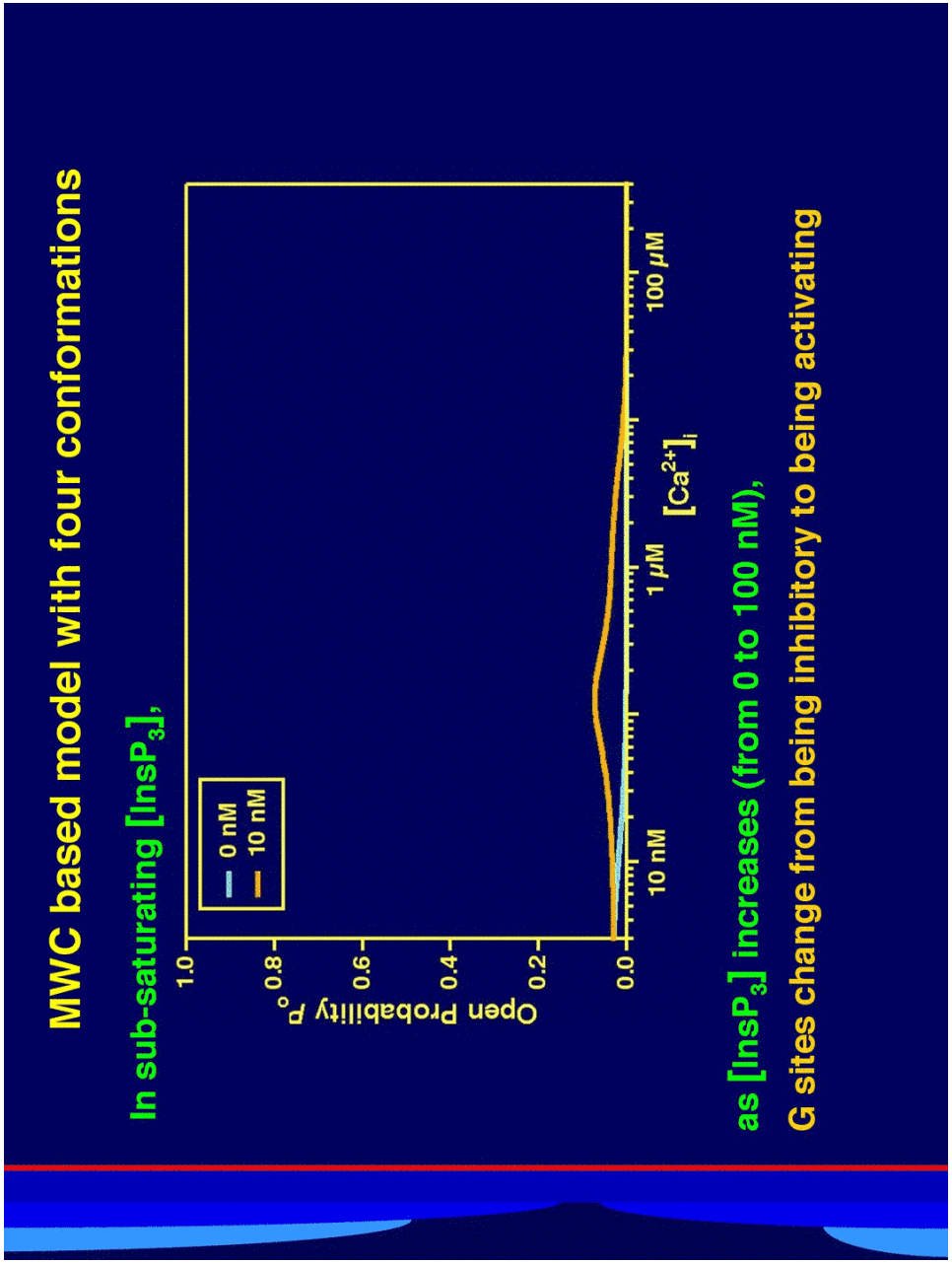
MWC based model with four conformations

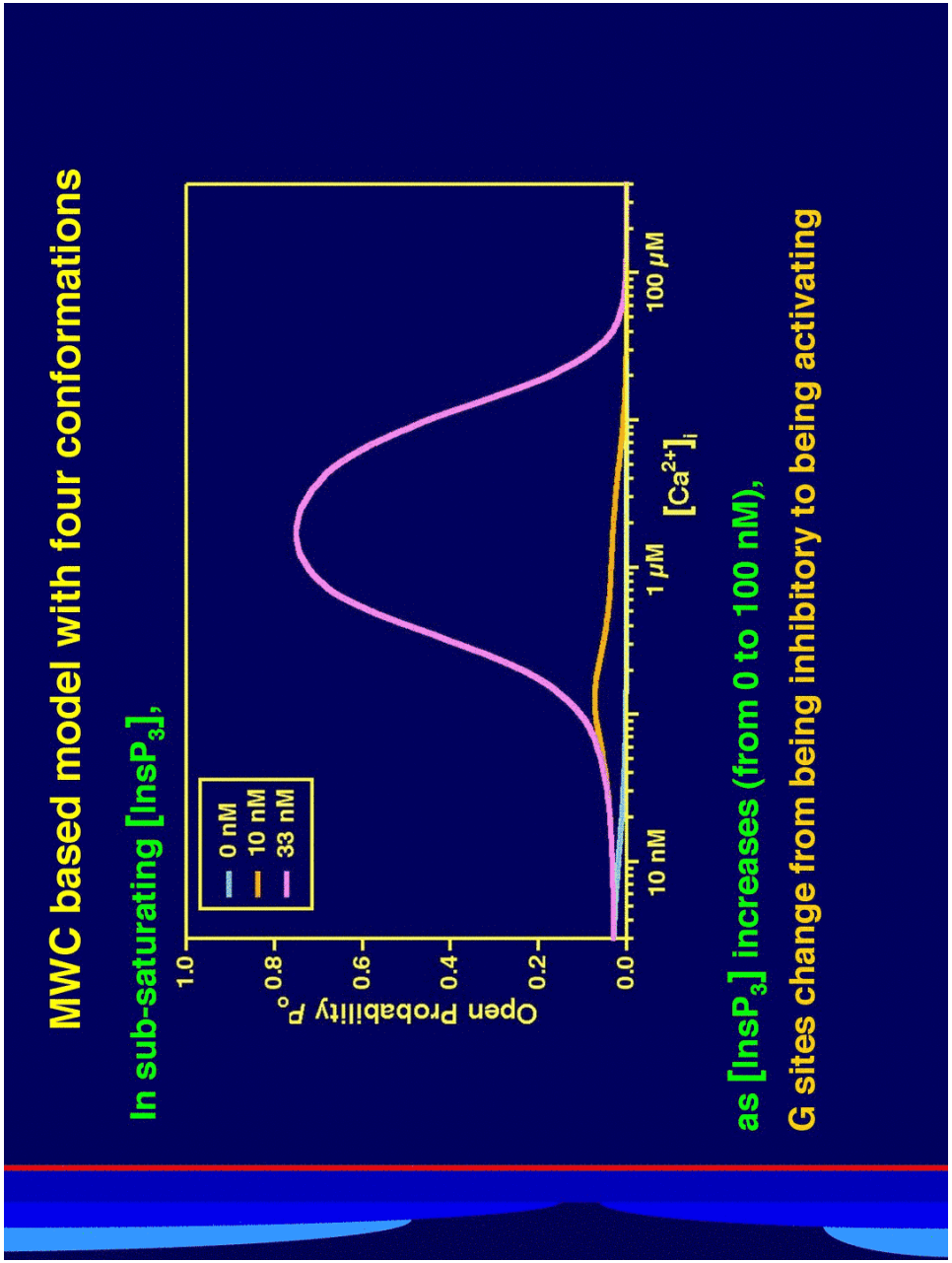
In sub-saturating [$InsP_3$],



as [$InsP_3$] increases (from 0 to 100 nM),

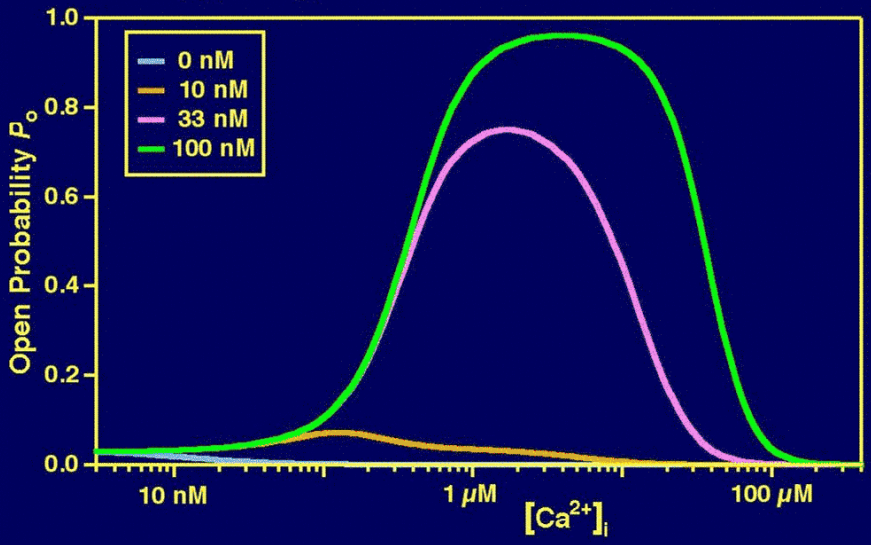
G sites change from being inhibitory to being activating


MWC based model with four conformations
In sub-saturating [InsP3],
Open Probability Po
1.0
0.8
0.6
0.4
0.2
0.0
0 nM
10 nM
10 nM
1 μM
100 μM
[Ca2+]i
as [InsP3] increases (from 0 to 100 nM),
G sites change from being inhibitory to being activating


MWC based model with four conformations
In sub-saturating [InsP3],
Open Probability Po
1.0
0.8
0.6
0.4
0.2
0.0
0 nM
10 nM
33 nM
10 nM
1 μM
100 μM
[Ca2+]i
as [InsP3] increases (from 0 to 100 nM),
G sites change from being inhibitory to being activating

MWC based model with four conformations

In sub-saturating [$InsP_3$],



as [$InsP_3$] increases (from 0 to 100 nM),

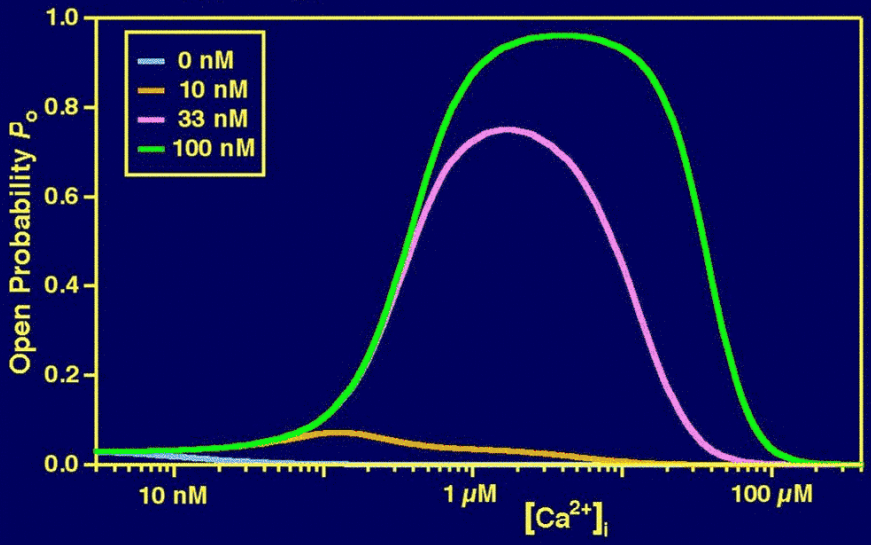
G sites change from being inhibitory to being activating

➔ Large change in Ca^{2+} response of the channel

➔ High sensitivity of the channel to [$InsP_3$] in a narrow range

MWC based model with four conformations

In sub-saturating [$InsP_3$],



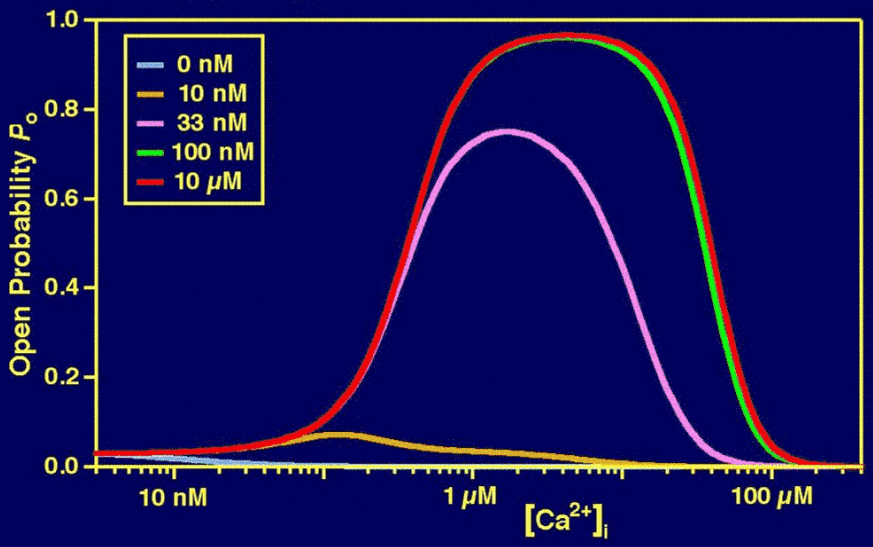
as G sites change from being inhibitory to being activating

➔ Effect of $InsP_3$-independent activating F sites is observable

➔ $InsP_3$ appears to affect mainly the K_{inh} of the channel, not K_{act} or H_{act} of the channel (which is determined by the F sites)

MWC based model with four conformations

In sub-saturating [$InsP_3$],



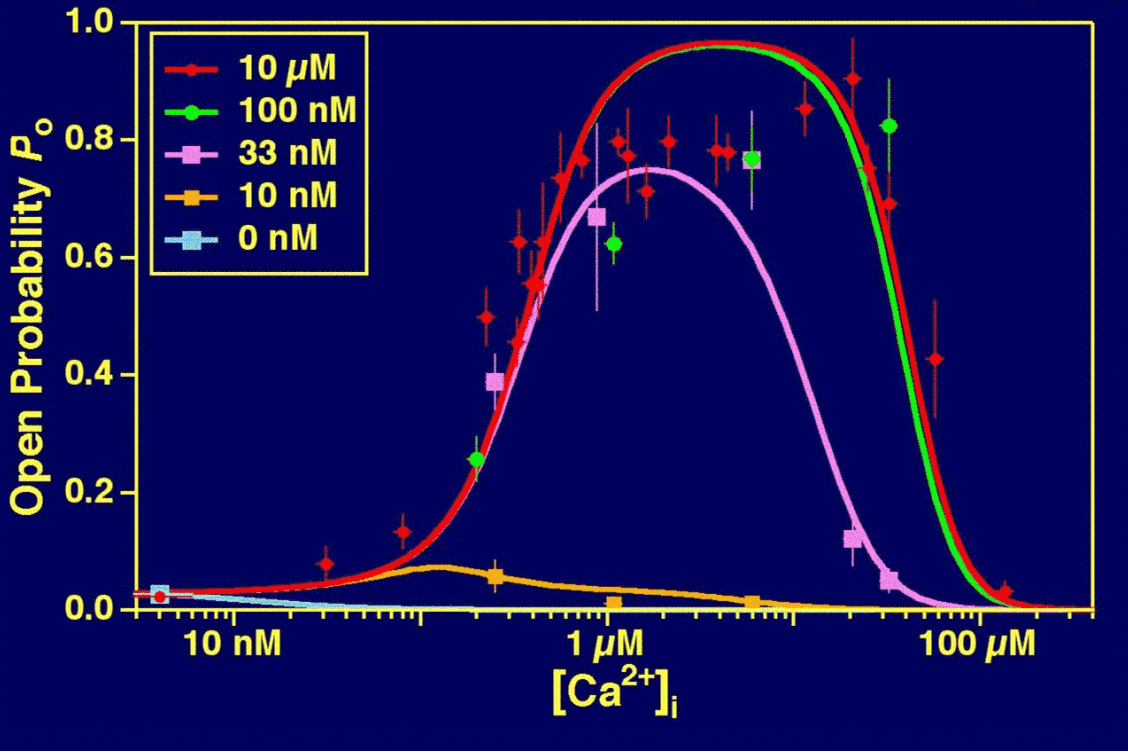
as G sites becomes activating

➔ Effect of low affinity $InsP_3$-independent inhibitory H sites can be observed at high $[Ca^{2+}]_i$

➔ $InsP_3$ regulation appears to saturate abruptly

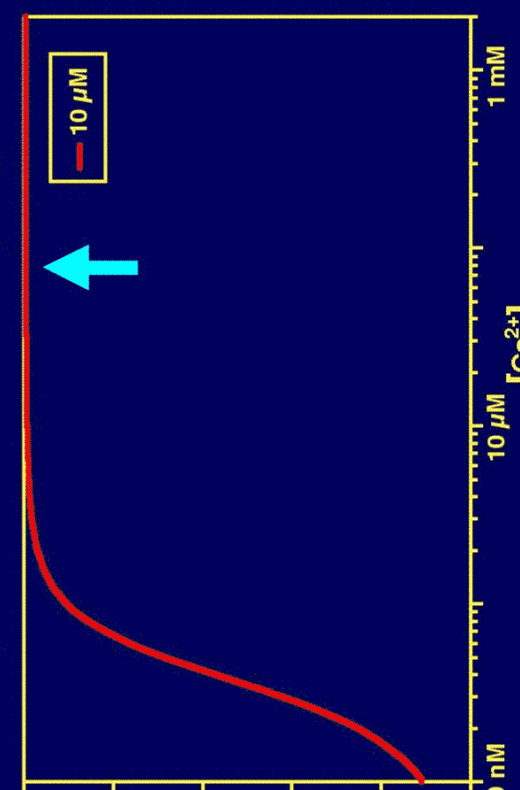
Fitting of experimental $InsP_3R$ channel P_o by MWC based model with four conformations

450 nM bath $[Ca^{2+}]$



MWC based model with four conformations

After channel is exposed to ultra-low bath $[Ca^{2+}]_i$,

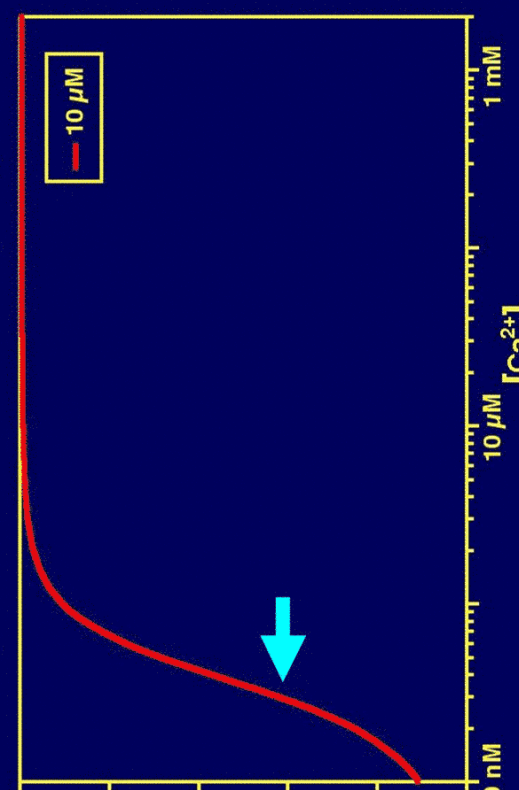


In the model, affinities of H sites in C* and D conformations become the same and the H sites are no longer functional

➔ Abrogation of high $[Ca^{2+}]_i$ inhibition mediated by H sites

MWC based model with four conformations

After channel is exposed to ultra-low bath $[Ca^{2+}]_i$,

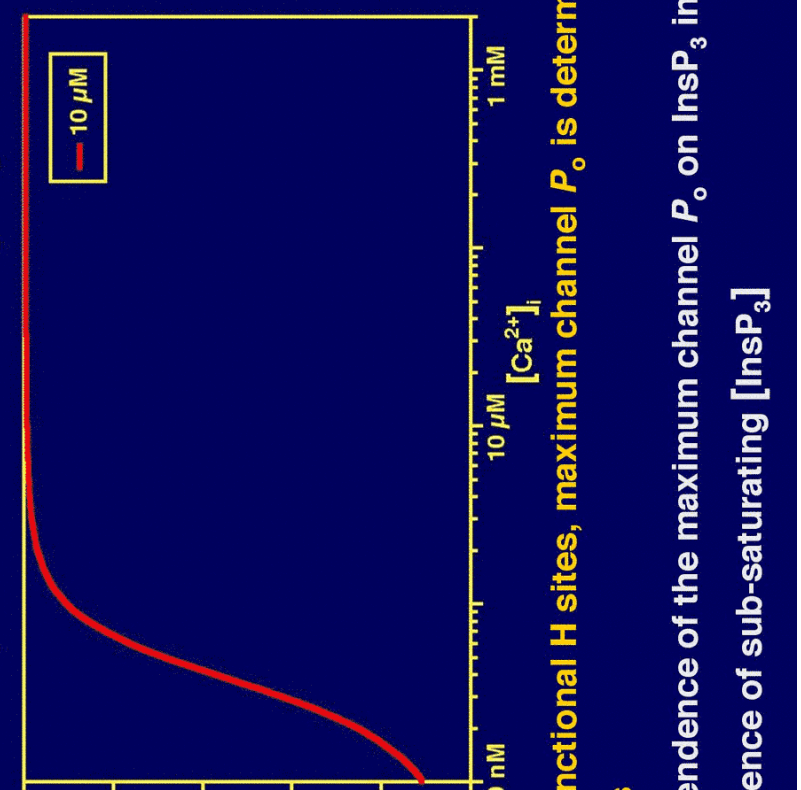


Affinities of the F and G sites are not affected

➔Similar Ca^{2+} activation of channels in saturating $[InsP_3]$ as that in channel exposed to 450 nM bath $[Ca^{2+}]$

MWC based model with four conformations

After channel is exposed to ultra-low bath $[Ca^{2+}]_i$,

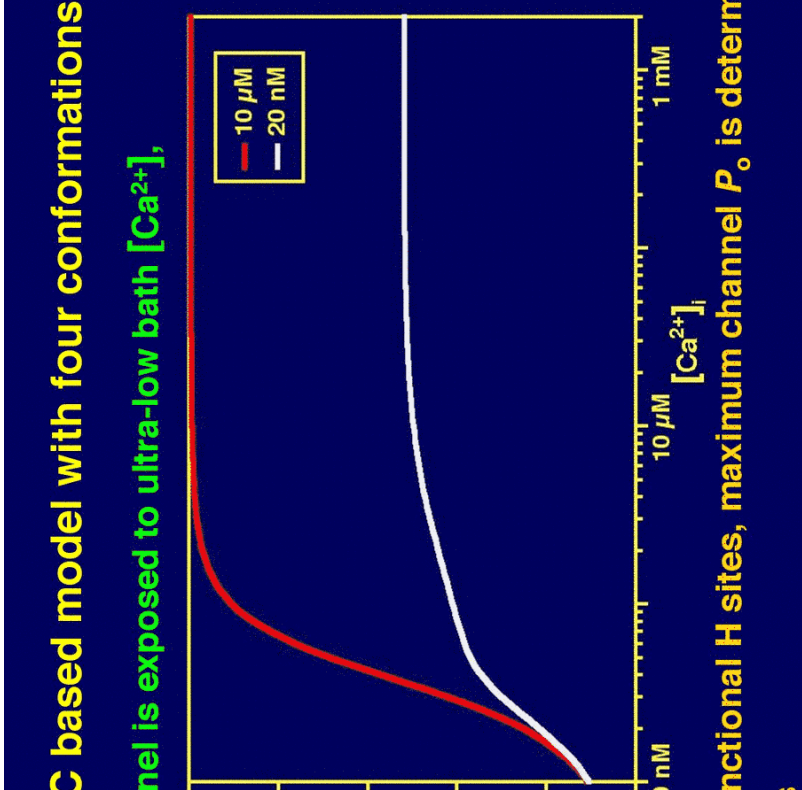


With no functional H sites, maximum channel P_o is determined by the G sites

➔ dependence of the maximum channel P_o on $InsP_3$ in the presence of sub-saturating $[InsP_3]$

MWC based model with four conformations

After channel is exposed to ultra-low bath $[Ca^{2+}]_i$,



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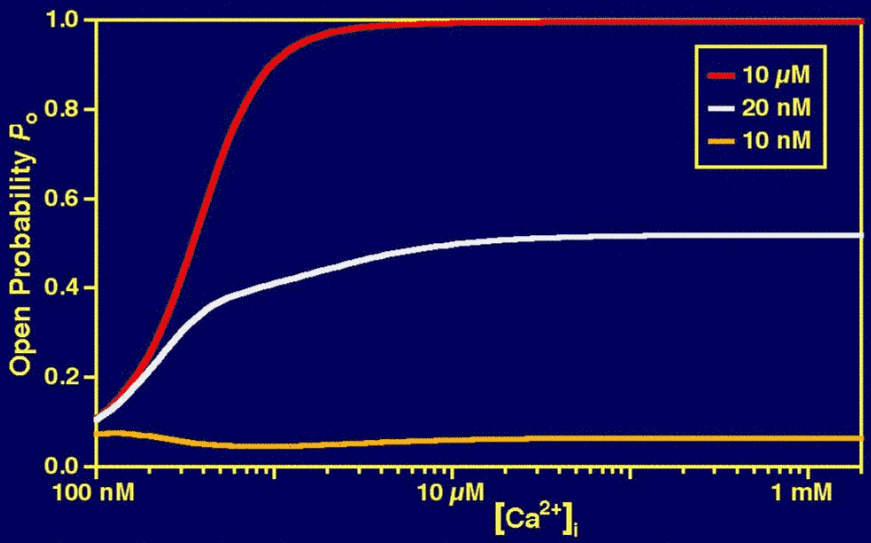
➔ dependence of the maximum channel P_o on $InsP_3$ in the presence of sub-saturating $[InsP_3]$

MWC based model with four conformations

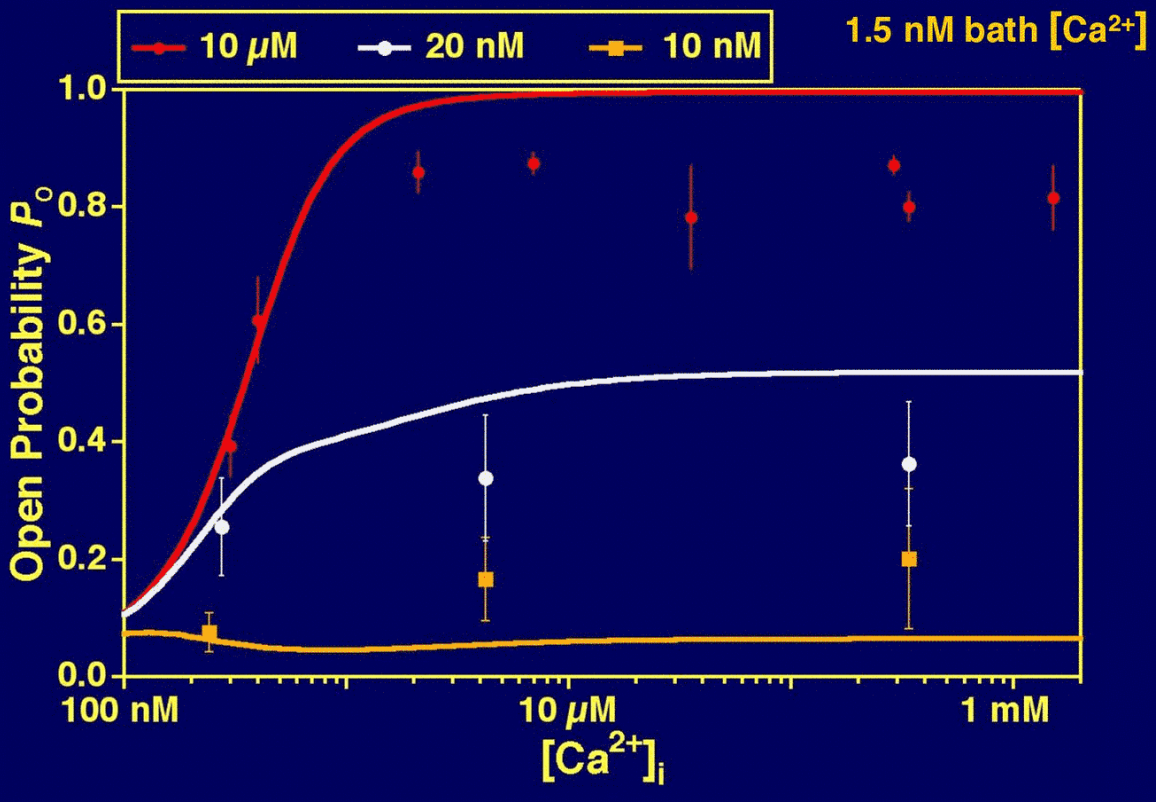
After channel is exposed to ultra-low bath $[Ca^{2+}]$,



With no functional H sites, maximum channel P_o is determined by the G sites

➔ dependence of the maximum channel P_o on $InsP_3$ in the presence of sub-saturating $[InsP_3]$

Fitting of experimental $InsP_3R$ channel P_o by MWC based model with four conformations

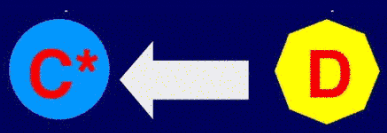


MWC based model with four conformations

Problem:

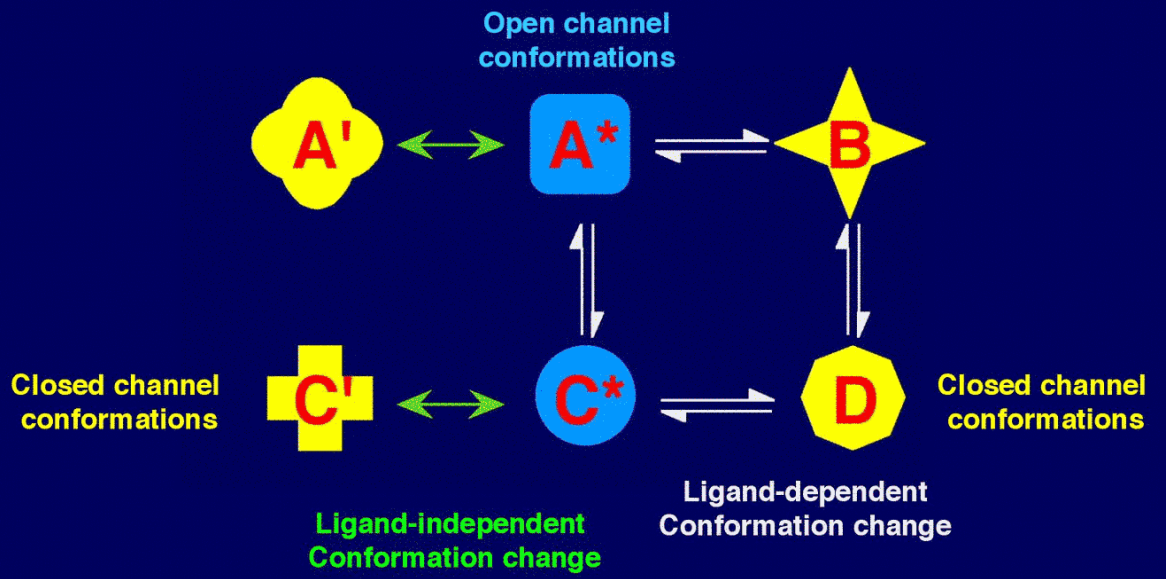
- After channel was exposed to ultra-low bath $[Ca^{2+}]$ (no high $[Ca^{2+}]_i$ inhibition as the H sites become non-functional),
- In saturating $[InsP_3]$ (both the F and G sites are activating)
- In high $[Ca^{2+}]_i$, (both F and G sites are bound)

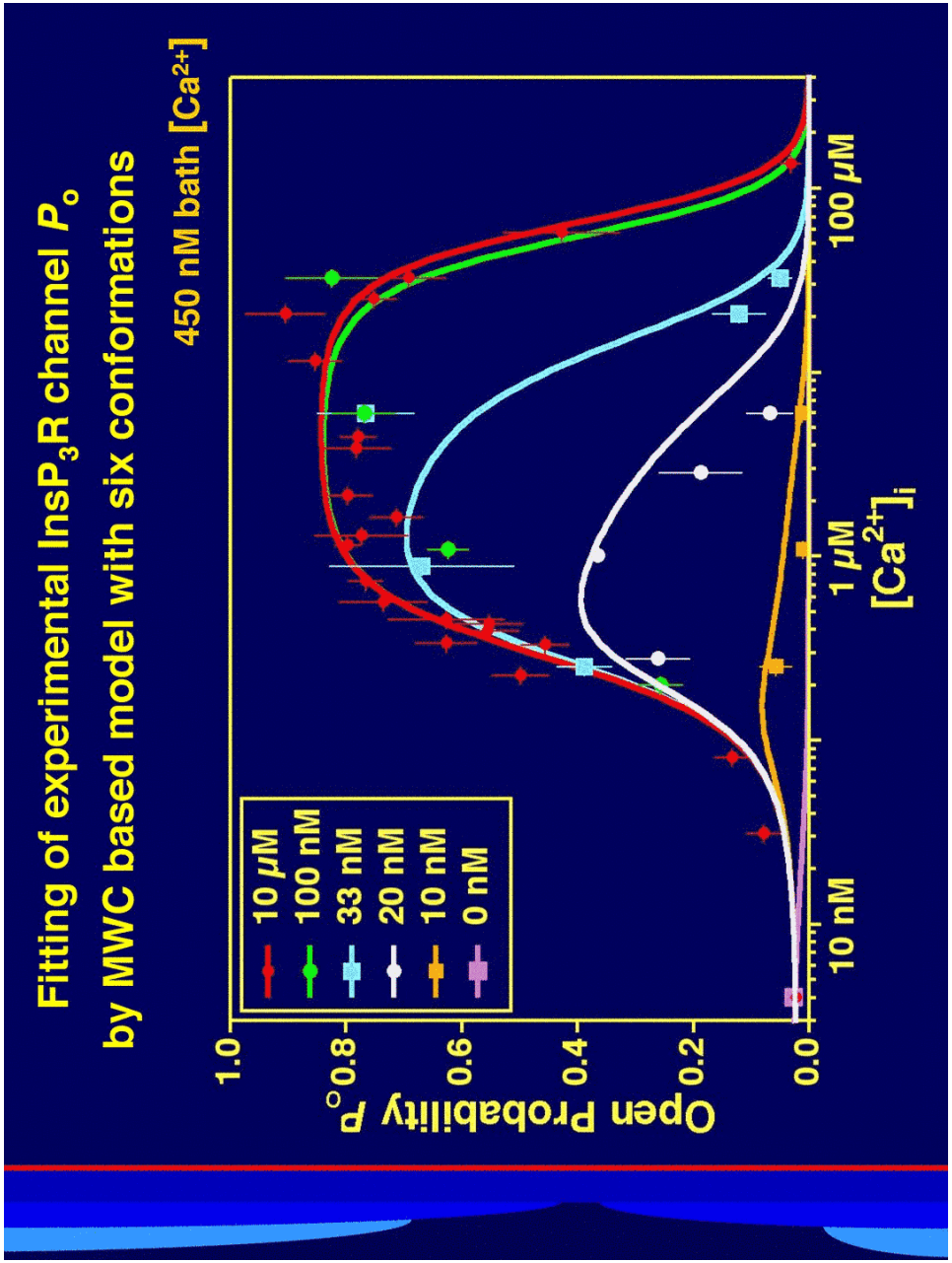
There is no inhibitory effect

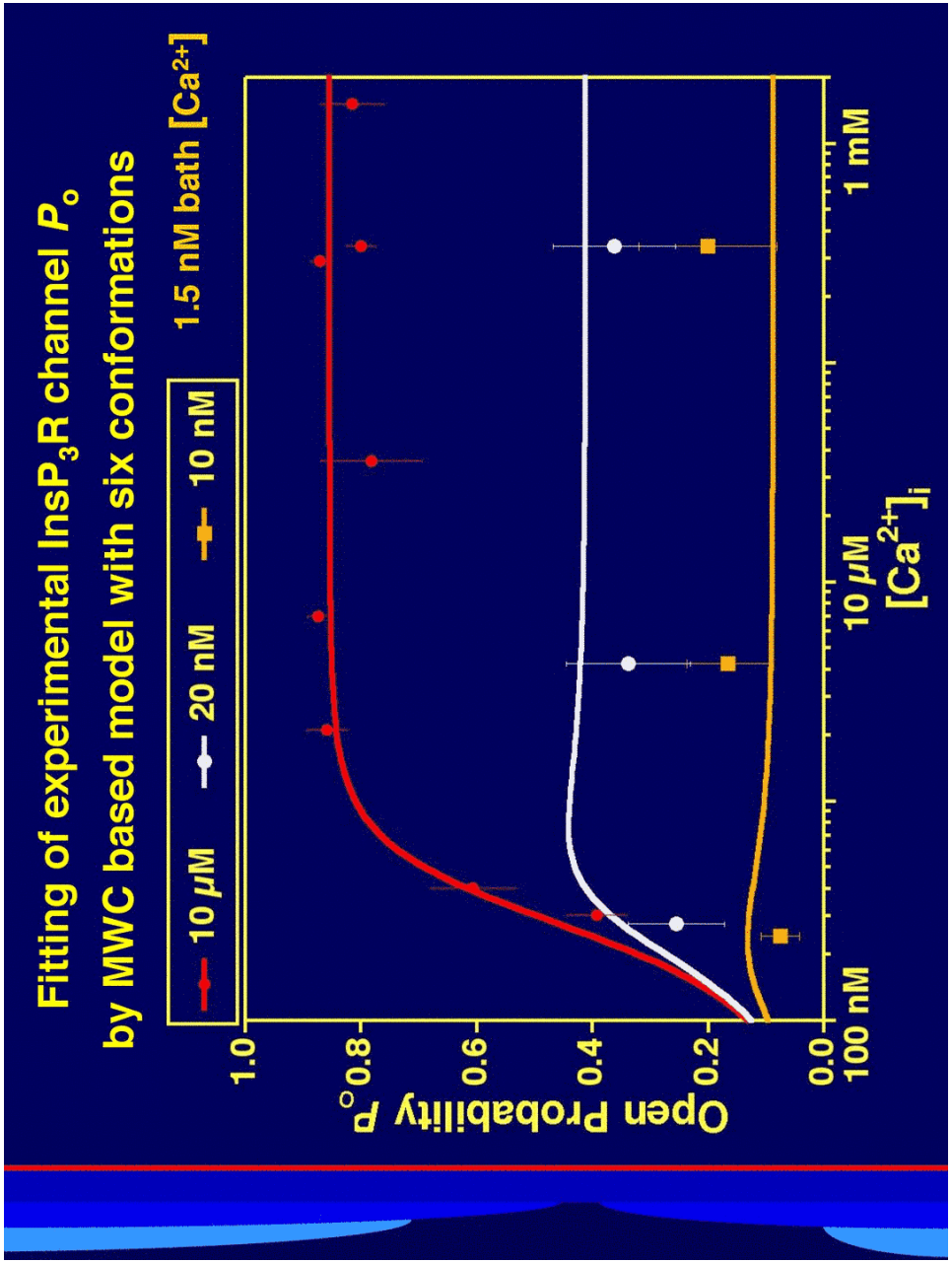


➔ Channel P_o will approach 1, *not* 0.8

MWC based model with six conformations




Fitting of experimental InsP3R channel Po
by MWC based model with six conformations
450 nM bath [Ca2+]
10 μM
100 nM
33 nM
20 nM
10 nM
0 nM
Open Probability Po
1.0
0.8
0.6
0.4
0.2
0.0
10 nM
1 μM
100 μM
[Ca2+]i


Fitting of experimental InsP3R channel Po
by MWC based model with six conformations
1.5 nM bath [Ca2+]
10 μM
20 nM
10 nM
Open Probability Po
1.0
0.8
0.6
0.4
0.2
0.0
100 nM
10 μM
1 mM
[Ca2+]i

Summary

» MWC-based six-conformation allosteric model

> The model successfully fit all the steady-state single-channel gating properties that have been observed:

– $[Ca^{2+}]_i$ regulation of the $InsP_3R$ channel

– $[InsP_3]$ regulation of the channel

– Spontaneous channel activity even when the channel is not bound to any ligand

– Disruption of high $[Ca^{2+}]_i$ inhibition of $InsP_3R$ gating

> The model also takes into account the tetrameric structure of the $InsP_3R$ channel

> The model indicates that whereas each $InsP_3R$ monomer has only one $InsP_3$-binding site, it has at least 3 distinct Ca^{2+}-binding sites, only one of which is $InsP_3$-dependent.