Mg^{2+}-dependent conformational change of RNA studied by fluorescence correlation and FRET on immobilized single molecules

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Contributed by Steven Chu, February 9, 2002

Fluorescence correlation spectroscopy (FCS) of fluorescence resonant energy transfer (FRET) on immobilized individual fluorophores was used to study the Mg^{2+}-facilitated conformational change of an RNA three-helix junction, a structural element that initiates the folding of the 30S ribosomal subunit. Transitions of the RNA ju

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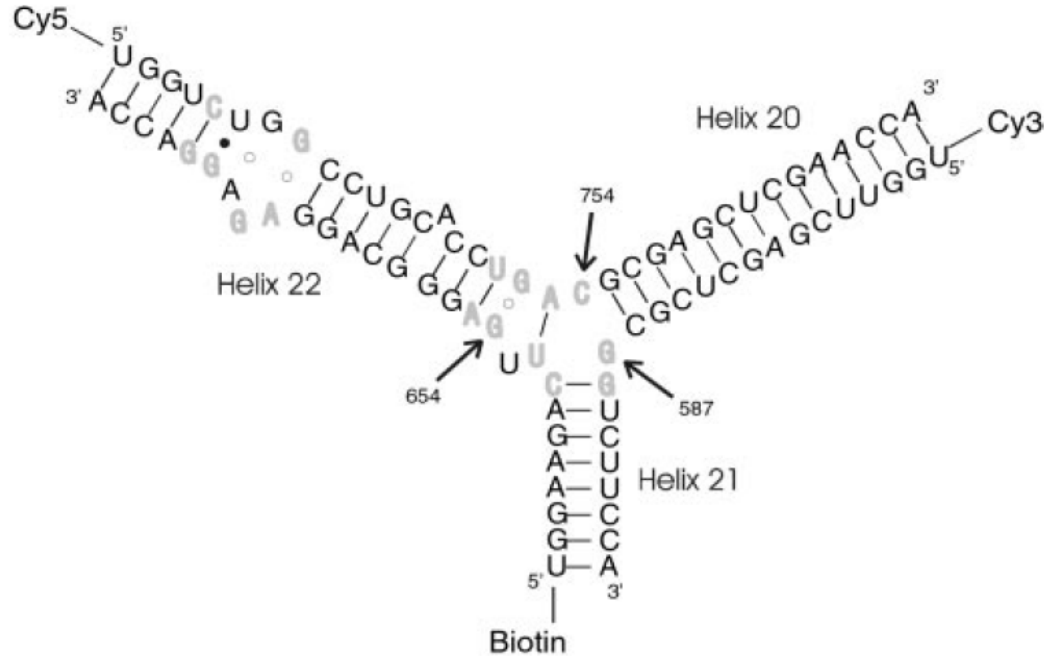
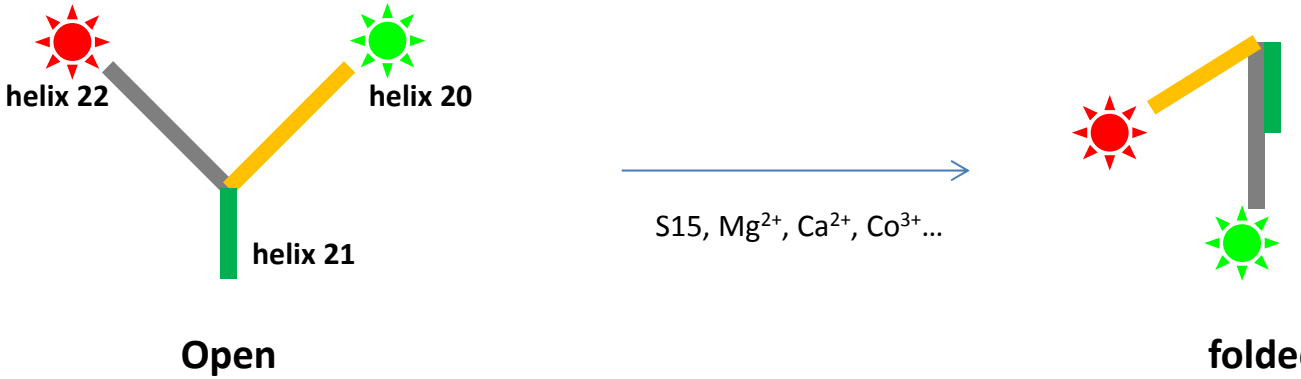
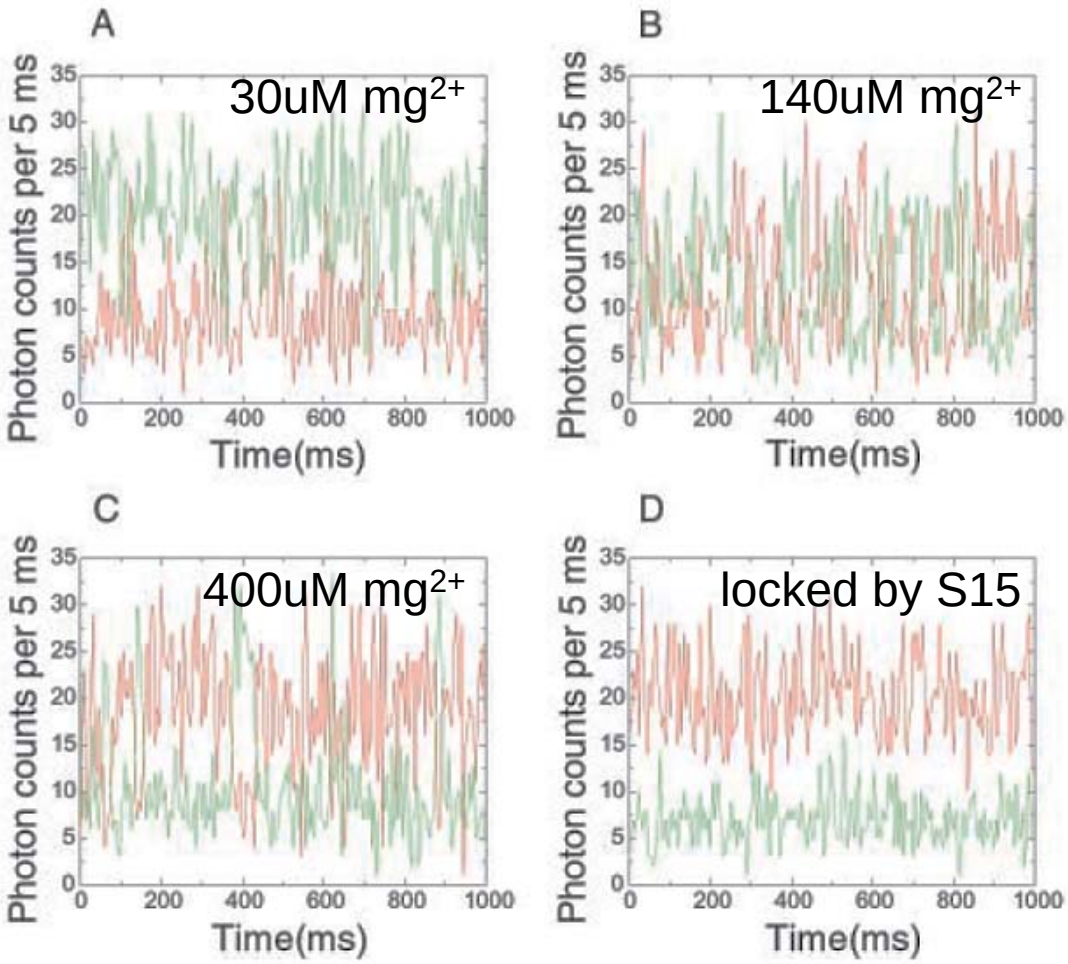
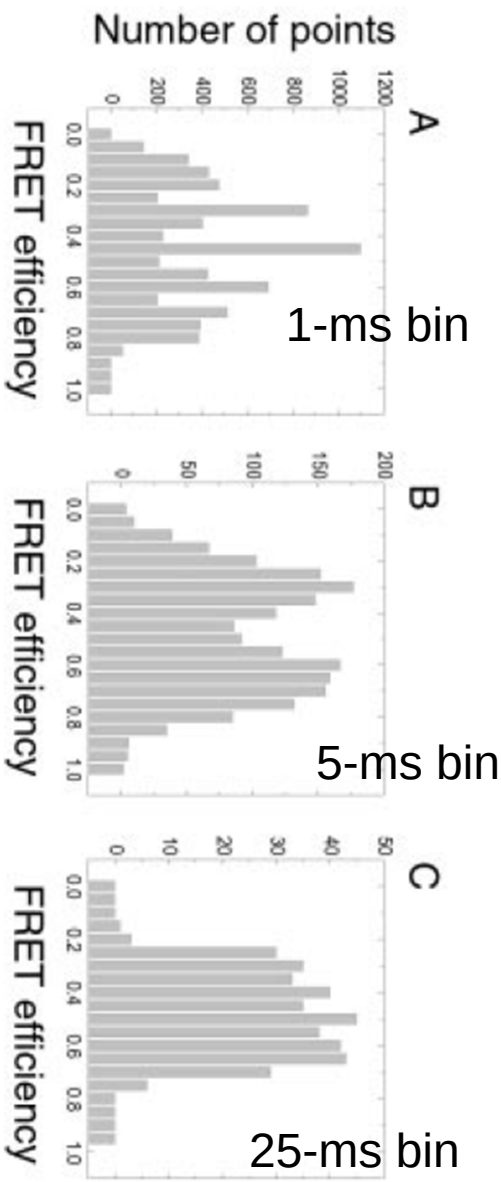
Ribosomal RNA






A
30uM mg2+
B
140uM mg2+
C
400uM mg2+
D
locked by S15
Photon counts per 5 ms
Time(ms)

140uM mg^{2+}


Number of points
FRET efficiency
A
1-ms bin
B
5-ms bin
C
25-ms bin

$$AC(\tau) = \frac{\langle \delta I(t)\delta I(t+\tau)\rangle}{\langle I(t)\rangle\langle I(t)\rangle} = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle\langle I(t)\rangle} - 1$$

$$CC(\tau) = \frac{\langle \delta I_d(t)\delta I_a(t+\tau)\rangle}{\langle I_d(t)\rangle\langle I_a(t)\rangle} = \frac{\langle I_d(t)I_a(t+\tau)\rangle}{\langle I_d(t)\rangle\langle I_a(t)\rangle} - 1$$

$$\frac{d}{dt}\begin{pmatrix} N_2(t) \\ N_1(t) \end{pmatrix} = \begin{pmatrix} -k_1 & k_2 \\ k_1 & -k_2 \end{pmatrix}\begin{pmatrix} N_2(t) \\ N_1(t) \end{pmatrix}$$

$$N_1(t) + N_2(t) = N$$

$$N_m(t) = \left(N_m(0) - \frac{k_m}{\lambda}N\right)e^{-\lambda t} + \frac{k_m}{\lambda}N$$

$$P(m) = \lim_{t\to\infty}\frac{N_m(t)}{N} = \frac{k_m}{\lambda} \qquad \lambda = k_1 + k_2$$

$$P(m, t|n, t+\tau) = \frac{N_n(t+\tau)}{N_m(t)} = \left(\delta_{mn} - \frac{k_n}{\lambda}\right)e^{-\lambda\tau} + \frac{k_n}{\lambda}$$

$N_m(t) = N$

$$\frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2} - 1 = \frac{\sum_{m=1}^{2}\sum_{n=1}^{2} I_m I_n P(m)P(m, t|n, t+\tau)}{\left(\sum_{m=1}^{2} I_m P(m)\right)^2} - 1$$

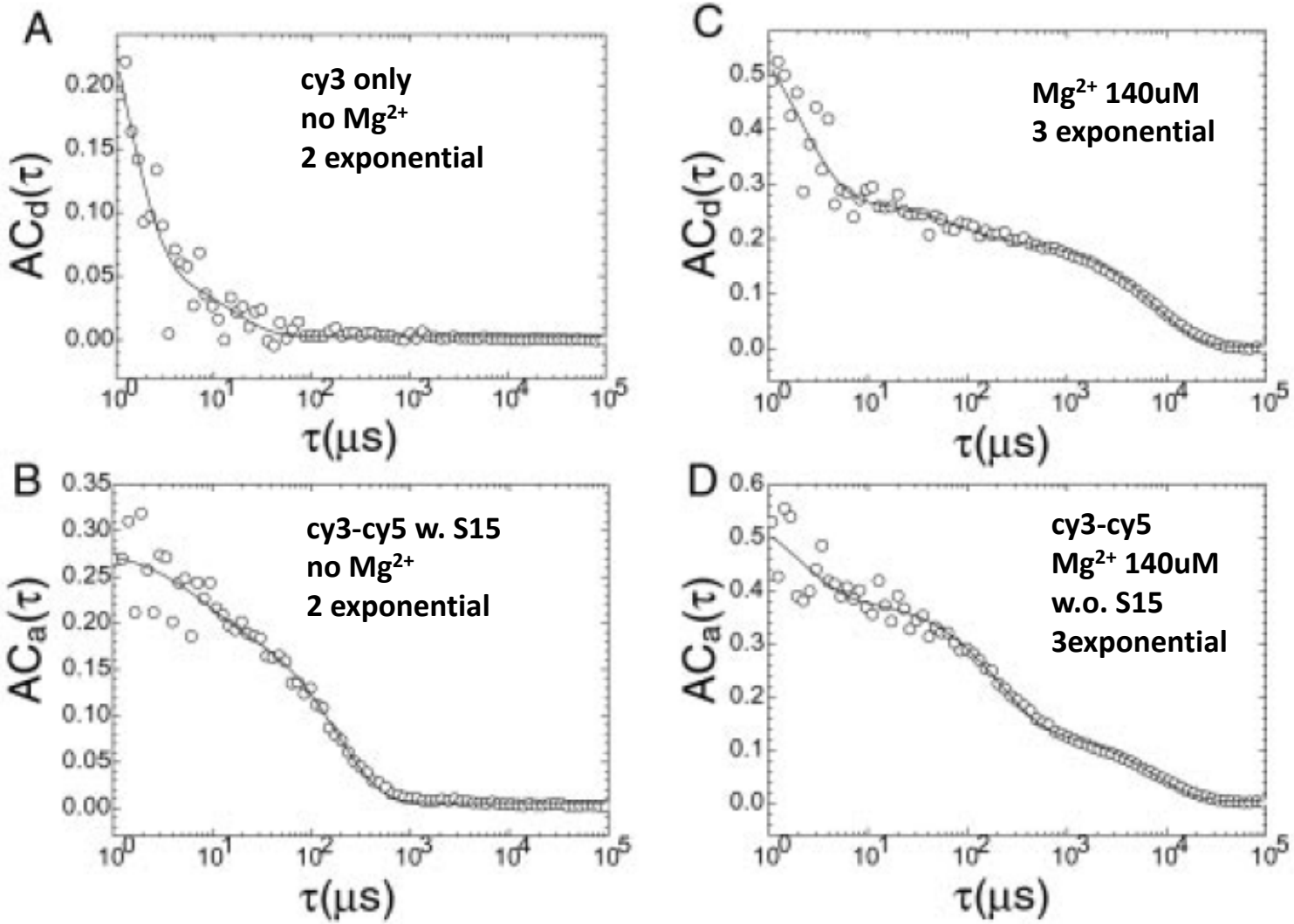
$$= \frac{(I_2 - I_1)^2 k_1 k_2}{(k_1 I_1 + k_2 I_2)^2}e^{-\lambda\tau}.$$

[

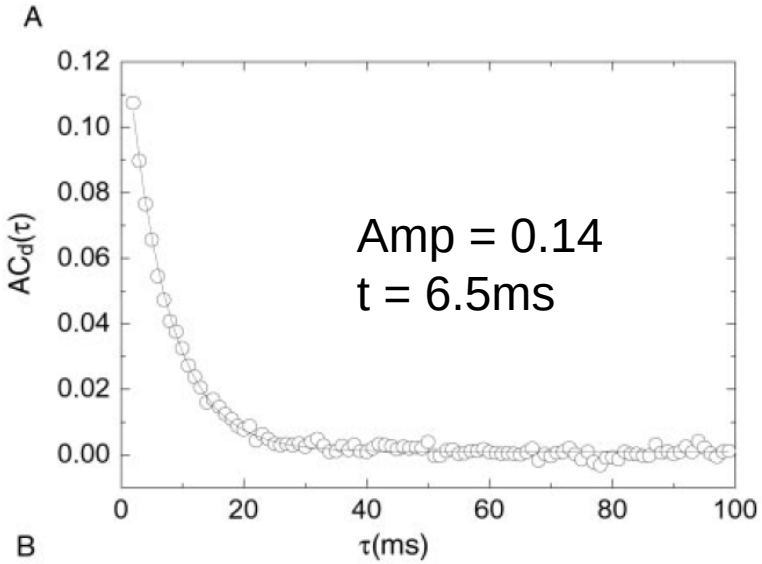
$$AC_a(\tau) = \frac{\left(\frac{I_a^{\text{folded}}}{I_a^{\text{open}}} - 1\right)^2}{\left(\frac{I_a^{\text{folded}}}{I_a^{\text{open}}} + \frac{k_{\text{o,obs}}}{k_{\text{f,obs}}}\right)^2} \frac{k_{\text{o,obs}}}{k_{\text{f,obs}}} e^{-\lambda\tau}$$

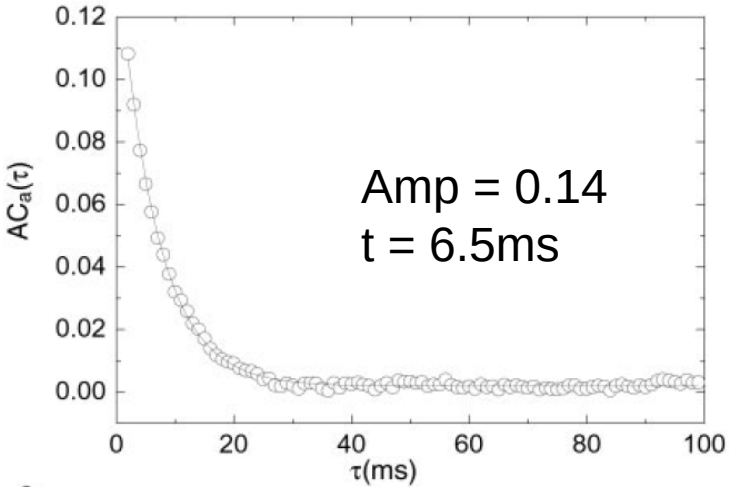
$$AC_d(\tau) = \frac{\left(\frac{I_d^{\text{open}}}{I_d^{\text{folded}}} - 1\right)^2}{\left(\frac{I_d^{\text{open}}}{I_d^{\text{folded}}} + \frac{k_{\text{f,obs}}}{k_{\text{o,obs}}}\right)^2} \frac{k_{\text{f,obs}}}{k_{\text{o,obs}}} e^{-\lambda\tau}$$

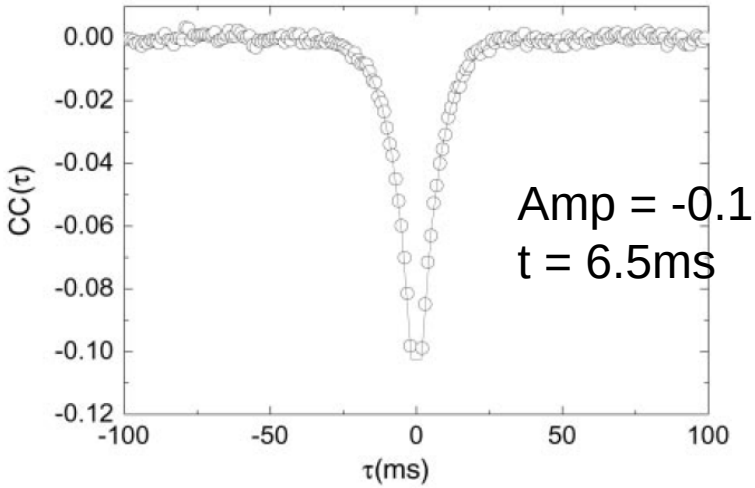
$$CC(\tau) = -[AC_a(\tau) \cdot AC_d(\tau)]^{1/2}$$


A
cy3 only
no Mg2+
2 exponential
ACd(τ)
τ(μs)
C
Mg2+ 140uM
3 exponential
ACd(τ)
τ(μs)
B
cy3-cy5 w. S15
no Mg2+
2 exponential
ACa(τ)
τ(μs)
D
cy3-cy5
Mg2+ 140uM
w.o. S15
3exponential
ACa(τ)
τ(μs)

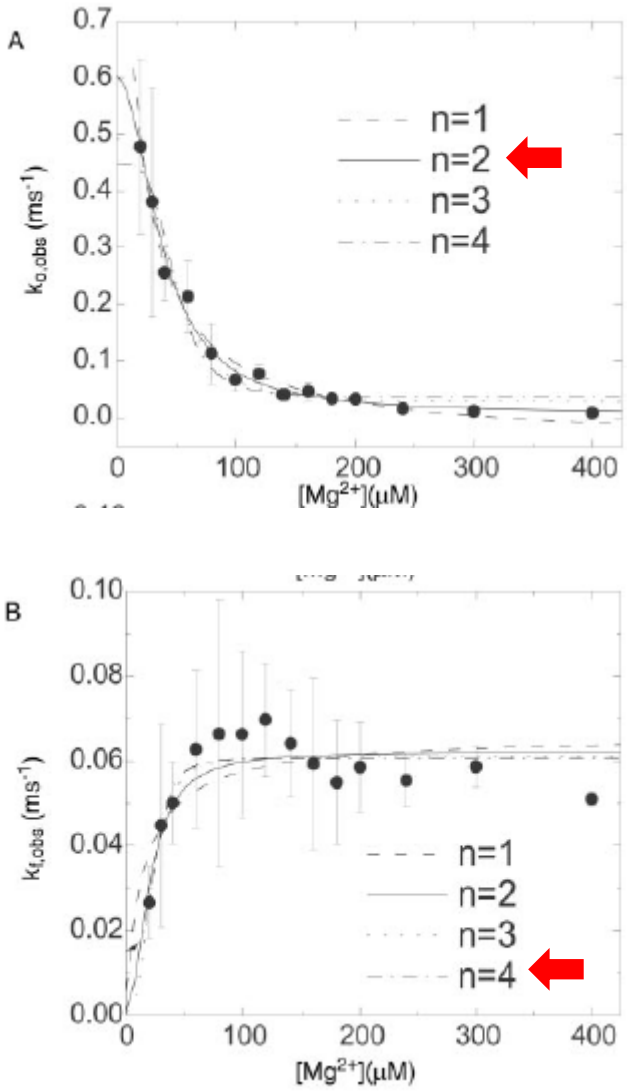
Correlation of 1 ms time bin







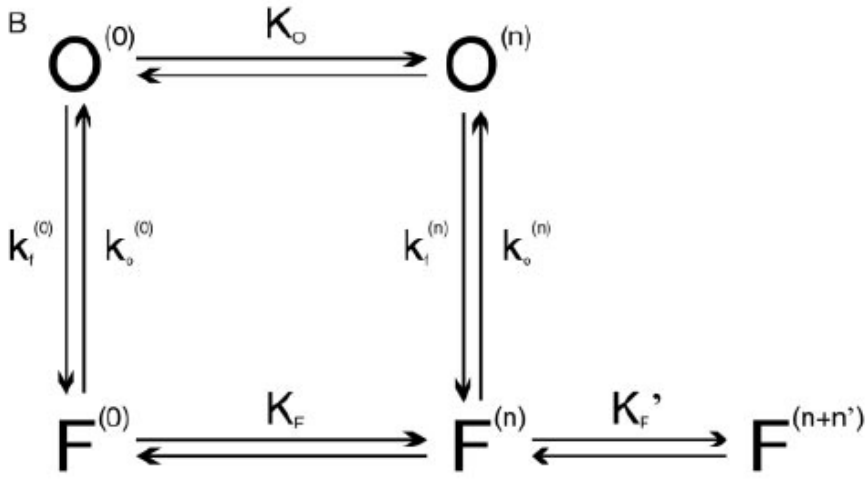
Mg^{2+} dependence



A

$$O^{(0)} + nMg^{2+} \rightleftharpoons F^{(n)}$$

B



$$k_{f,obs} = \frac{k_f^{(0)} + k_f^{(n)} \cdot (K_o[Mg^{2+}])^n}{1 + (K_o[Mg^{2+}])^n}$$

$$k_{o,obs} = \frac{k_o^{(0)} + k_o^{(n)} \cdot (K_F[Mg^{2+}])^n}{1 + (K_F[Mg^{2+}])^n}$$

Na^+ dependence