

# Mg<sup>2+</sup>-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<sup>2+</sup>-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 junction were found to occur on a timescale of milliseconds. Correlation analysis of FRET transitions revealed that Mg<sup>2+</sup> or Na<sup>+</sup> were found to facilitate the observed conformational change. Induced by Mg<sup>2+</sup>, the junction transition was modeled in a two-state model. In the junction transition, FCS/FRET analysis revealed a powerful biomolecular tool for studying biomolecular dynamics on the millisecond timescale.

showed that various cations such as Mg<sup>2+</sup>, Ca<sup>2+</sup>, Co<sup>3+</sup>, and spermidine alone also yield the same folded conformation of the junction (8, 9). Crystallographic studies located 8 Mg<sup>2+</sup> ions around the junction region that may be involved in stabilizing the folded form (10).

## Mg<sup>2+</sup>-dependent conformational change of RNA studied by fluorescence correlation and FRET on immobilized single molecules

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Monodisperse RNA structures have been studied using a variety of techniques including tRNA (2), hammerhead ribozyme (3), the P4-P6 domain of the *Tetrahymena thermophila* group I intron (4), and a 5S ribosomal RNA domain (5). Metal ions can also stabilize the RNA structure nonspecifically by screening the negatively charged backbone (6). Furthermore, the role of counterions is critical to understanding protein-nucleic acid interactions (7).

In the case of RNA-protein interactions, the situation can be even more complex when ion-dependent conformational changes accompany protein binding. An interesting example is the three-helix junction located in the central core of the 30S ribosomal RNA that is the binding site for the 16S ribosomal protein S15 (8). Biochemical analysis of the binding of S15 to the 30S subunit of *T. thermophila* has shown that a large conformational change occurs in the junction region (8, 9). Two different models have been proposed for the structure of the ribonucleoprotein complex including the junction region and S15 protein. The structure of the entire 30S subunit has been determined in the open (unfolded) form of the junction (10). Helices 20, 21, and 22 are arranged with nearly no interaction between them. The folded form of the junction is induced by the presence of ions or upon the binding of S15, which is located coaxially under helix 22, and helix 20 makes contact with helix 22. This unusual structure is stabilized in part by base-pairing between C754 and G654. Structural studies based on gel mobility of this RNA

showed that various cations such as Mg<sup>2+</sup>, Ca<sup>2+</sup>, Co<sup>3+</sup>, and spermidine alone also yield the same folded conformation of the junction (8, 9). Crystallographic studies located 8 Mg<sup>2+</sup> ions around the junction region that may be involved in stabilizing the folded form (10).

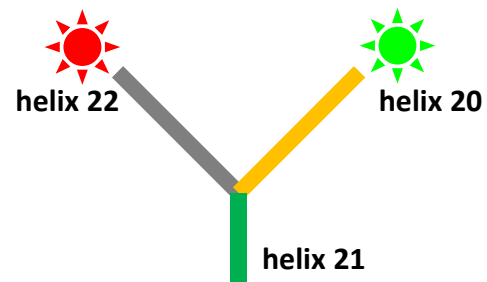
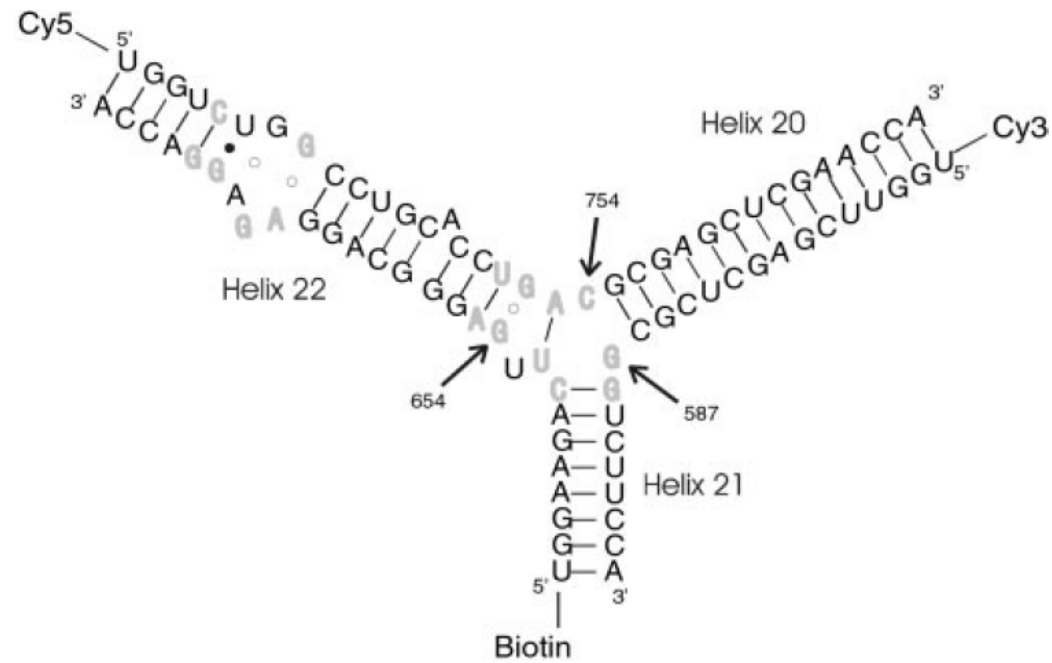
### Materials and Methods

**Sample Preparation.** A Cy3-Cy5 donor-acceptor pair was attached to two ends of the three-helix junction (Fig. 1), and a biotin moiety attached to the third helix was used for immobilization on a streptavidin-coated surface.

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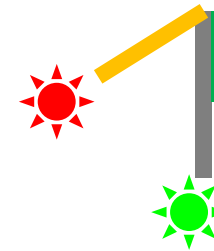
# Ribosomal RNA



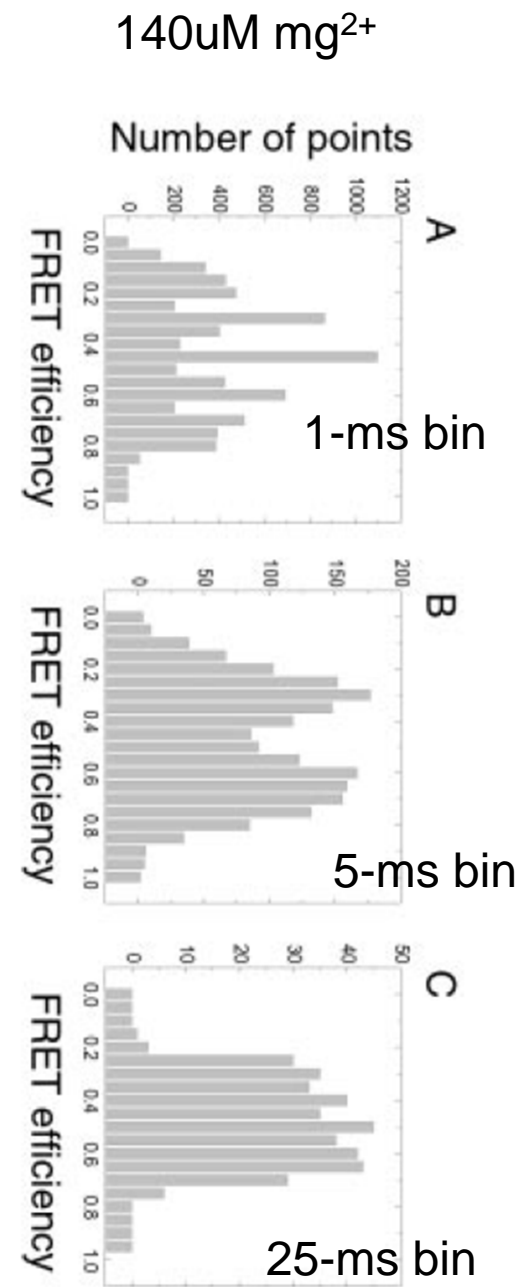
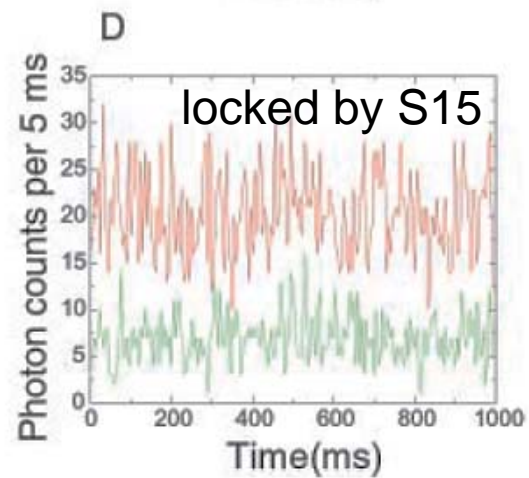
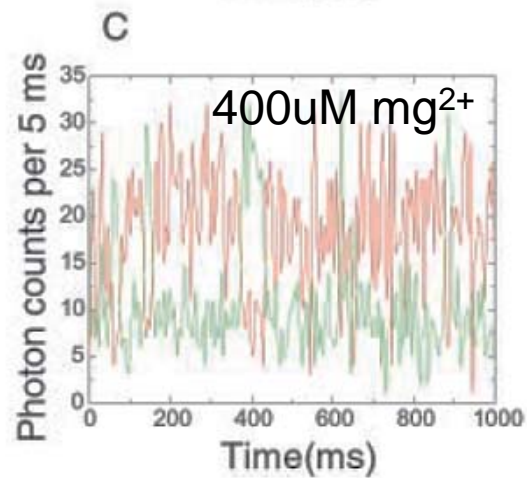
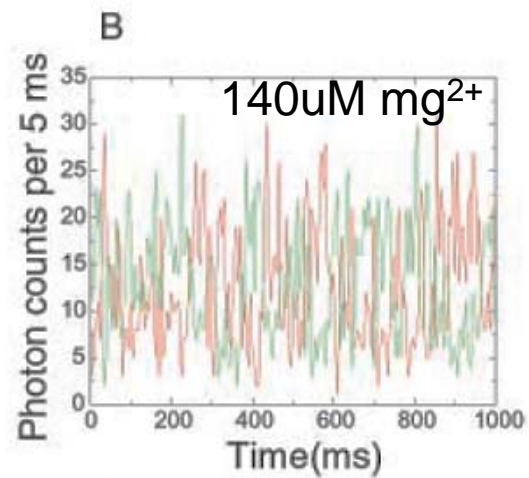
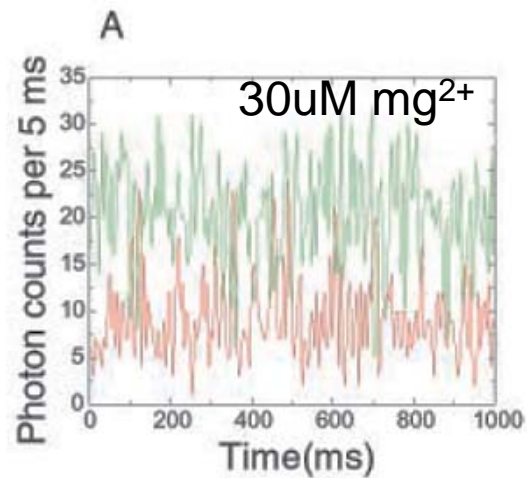
Open



S15,  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Co^{3+}$ ...



folded



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

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

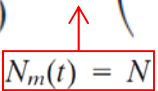
$$CC(\tau) = \frac{\langle \delta I_d(t) \delta I_a(t + \tau) \rangle}{\langle I_d(t) \rangle \langle I_a(t) \rangle} = \frac{\langle I_d(t) I_a(t + \tau) \rangle}{\langle I_d(t) \rangle \langle I_a(t) \rangle} - 1$$

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

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

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

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



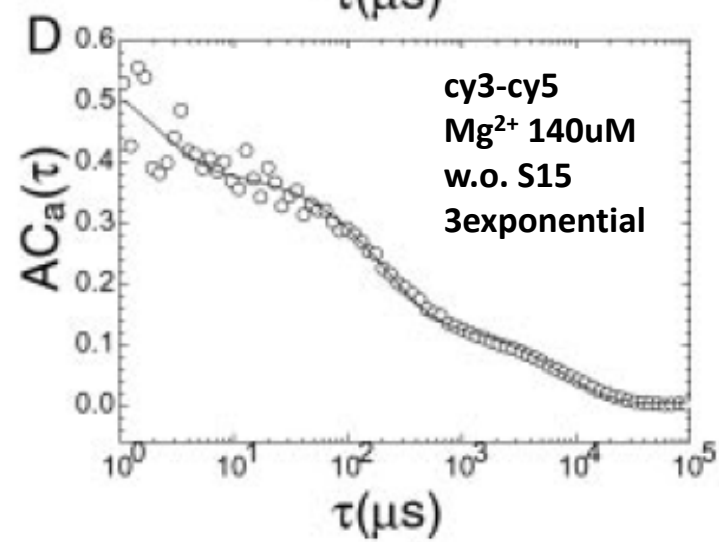
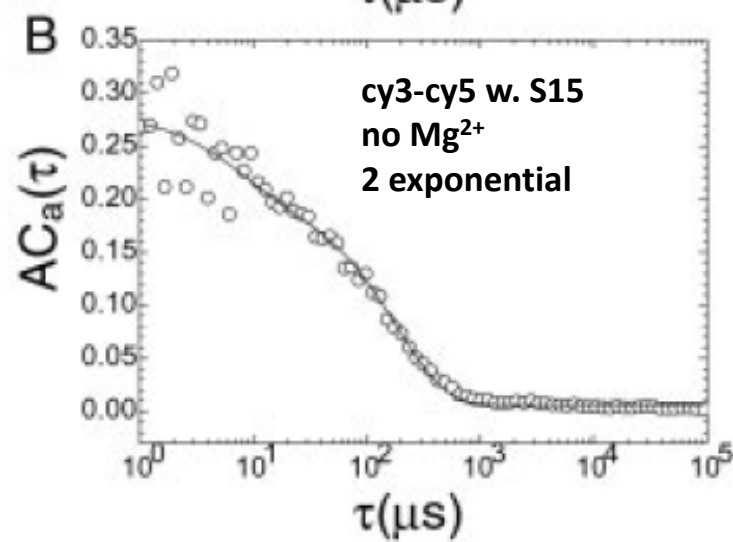
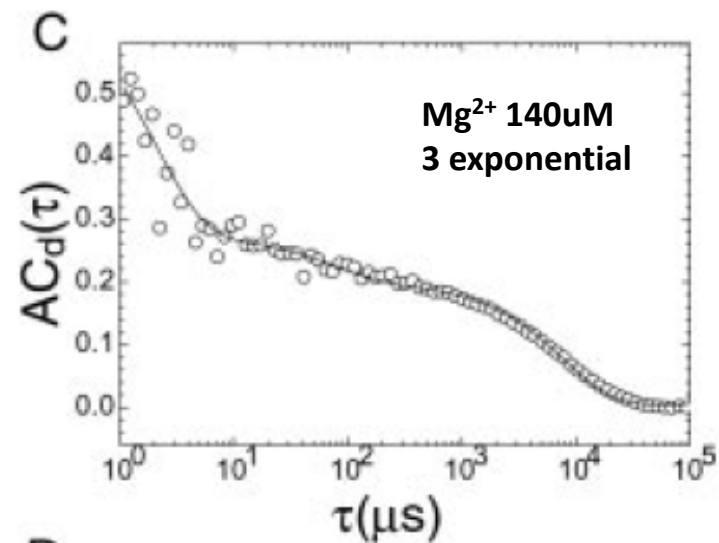
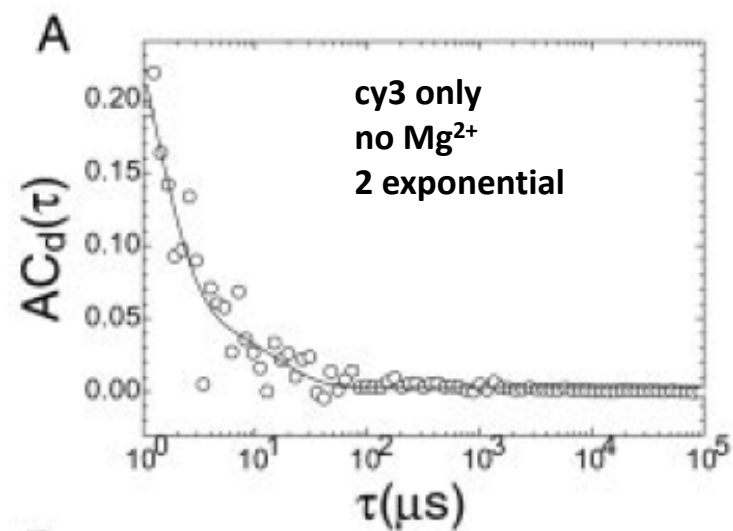
$$\frac{\langle I(t) I(t + \tau) \rangle}{\langle I(t) \rangle^2} - 1 = \frac{\sum_{m=1}^2 \sum_{n=1}^2 I_m I_n P(m) P(m, t|n, t + \tau)}{\left( \sum_{m=1}^2 I_m P(m) \right)^2} - 1$$

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

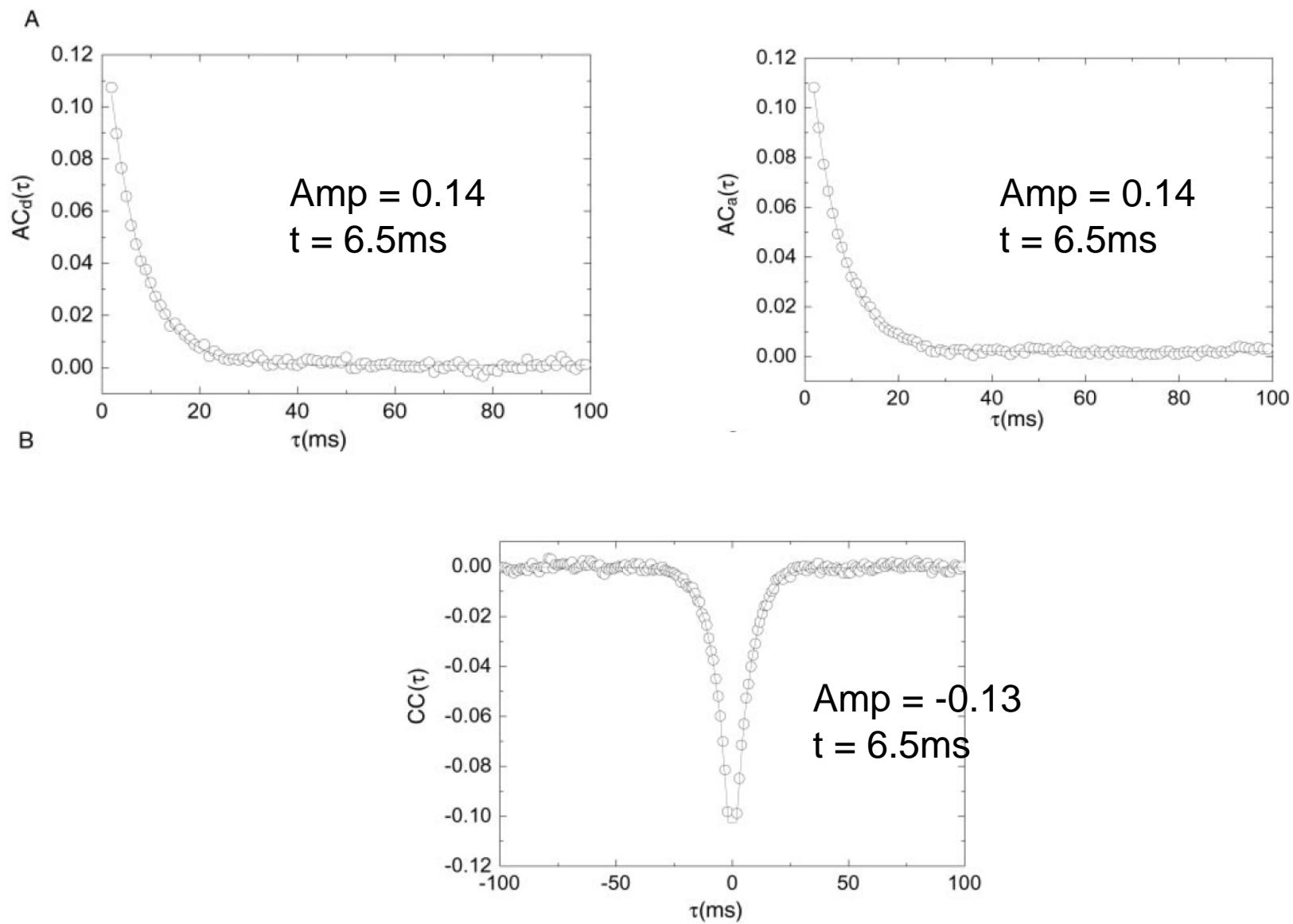
$$AC_a(\tau) = \frac{\left(\frac{I_a^{\text{folded}}}{I_a^{\text{open}}} - 1\right)^2}{\left(\frac{I_a^{\text{folded}}}{I_a^{\text{open}}} + \frac{k_{\text{o,obs}}}{k_{\text{f,obs}}}\right)^2} \frac{k_{\text{o,obs}}}{k_{\text{f,obs}}} e^{-\lambda\tau}$$

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

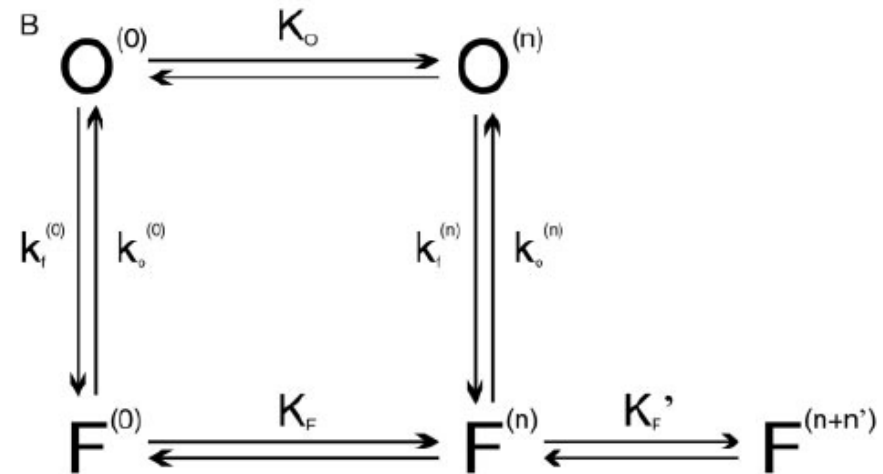
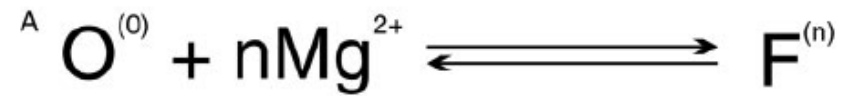
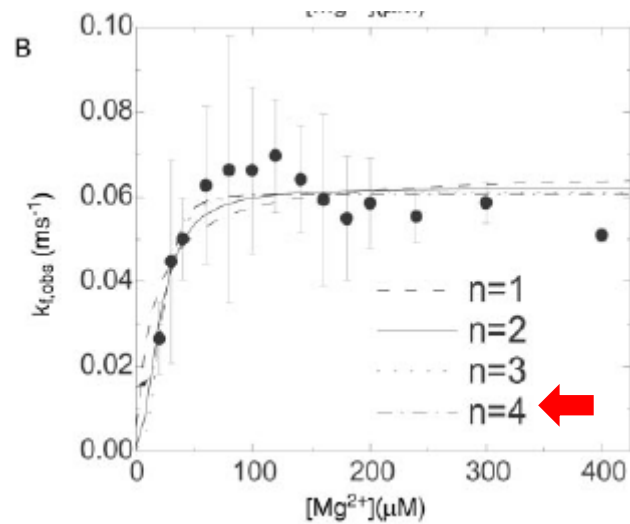
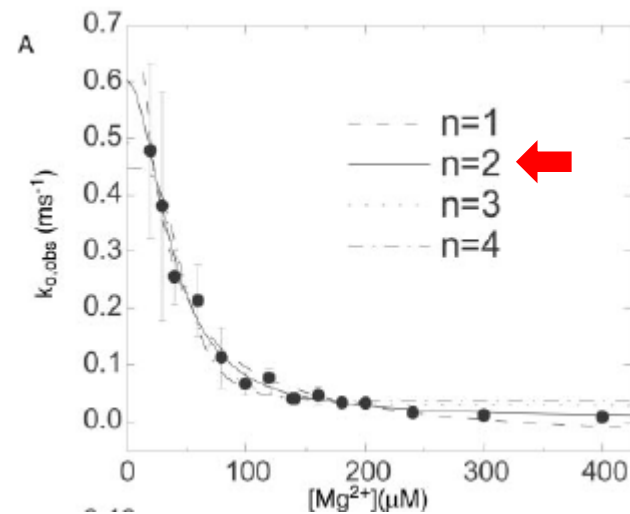
$$CC(\tau) = -[AC_a(\tau) \cdot AC_d(\tau)]^{1/2}$$



## Correlation of 1 ms time bin



## Mg<sup>2+</sup> dependence



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

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





## Na<sup>+</sup> dependence

