IκBα Structure and Dynamics Explored by smFRET

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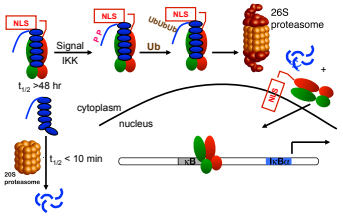
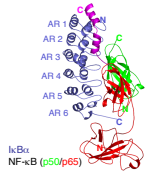
Introduction

More than 150 target genes, involved in a wide variety of cellular functions, are regulated by the nuclear factor kappa B (NF-κB) transcription factors. NF-κB is induced by many classes of stimuli, and it plays a key role in the regulation of cellular development and proliferation and in the immune and inflammatory responses. Aberrant regulation of NF-κB has been implicated in a wide variety of disease states, including cancer, heart disease, AIDS, Alzheimer's disease, and arthritis.



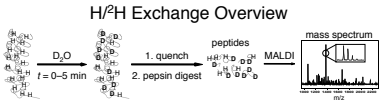
NF-κB transcriptional activity is regulated by the IκB inhibitor proteins. In resting cells, IκBα sequesters NF-κB in the cytosol. Upon stimulation, IκB kinase (IKK) phosphorylates IκB, which initiates the ubiquitination and degradation of IκB by the 26S proteasome. Free NF-κB translocates to the nucleus, binds to DNA, and activates transcription of its many target genes, including IκBα. Newly synthesized IκBα enters the nucleus, binds to NF-κB and the NF-κB•IκBα complex is exported to the cytosol, returning the cell to its resting state. Free IκBα levels are regulated by its basal degradation by the 20S proteasome, which does not degrade NF-κB-bound IκBα.

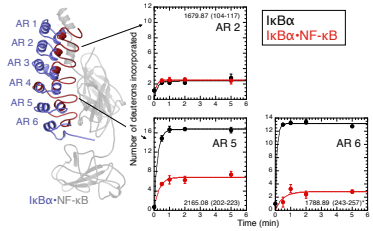
Crystal structure of IκBα provides only a static view when bound to NF-κB



The crystal structure of IκBα (blue) bound to NF-κB (p50, green and p65, red; p65 NLS and flanking helices are magenta). IκBα contains six ankyrin repeats (AR), a ~33-amino-acid structural motif that generally adopts a helix-loop-helix-β-hairpin/loop fold. The p65 NLS and flanking helices (magenta) interact with the first three ankyrin repeats (ARs 1–3) of IκBα. The dimerization domains of both p50 and p65 form an extensive interface with ARs 3–6 and also form additional contacts with the C-terminal PEST sequence of IκBα. The p65 N-terminal domain also makes additional contacts with the C-terminal IκBα PEST sequence. The N-terminal domain of p50 is not involved in IκBα binding.

β-hairpins in ARs 5–6 show large decreases in solvent accessibility when bound to NF-κB

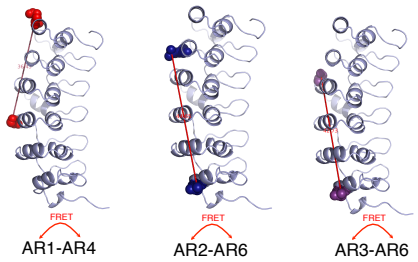




Truhlar, S. M.; Torpey, J. W.; Komives, E. A. *PNAS* **2006**, *103*, 18951-6.

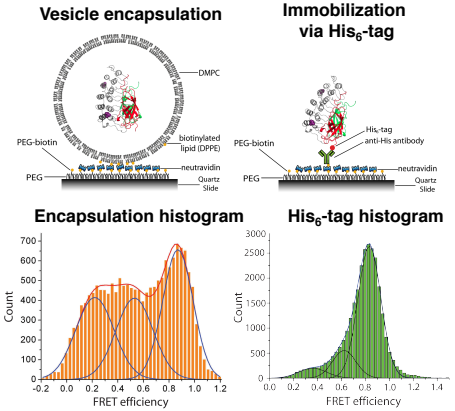
Engineered IκBα sites for conjugation of FRET fluorophores

Single cysteines were introduced in each ankyrin repeat (using a Cys-free IκBα template) for conjugation with thiol-reactive FRET fluorophores.

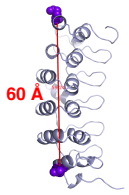


IκBα immobilization approaches for smFRET

Interaction with DMPC lipids seem to partially unfold IκBα AR 3-6.



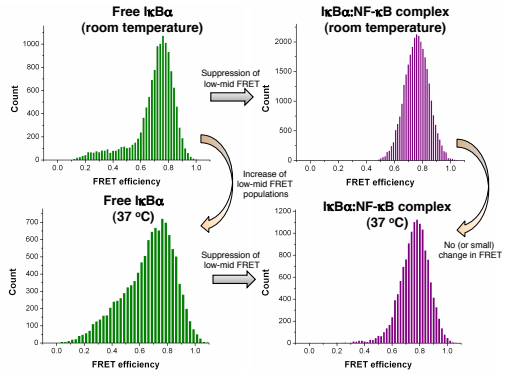
Cy3/Cy5 vs. Alexa 555/Alexa 647 dyes



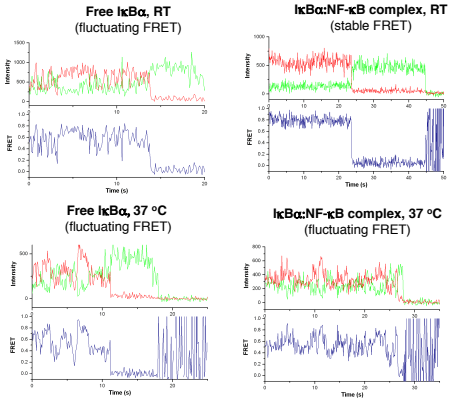
Preliminary studies with the Cy3/Cy5 pair (R_0 = **~60 Å**) revealed saturated FRET levels for several IκBα constructs, and observation of IκBα fluctuations was rare with this pair. The Alexa 555/647 pair provided a shorter R_0 (**~51 Å**), which improved the FRET levels and allowed for resolution of IκBα fluctuations.

N-terminally His-tagged IκBα

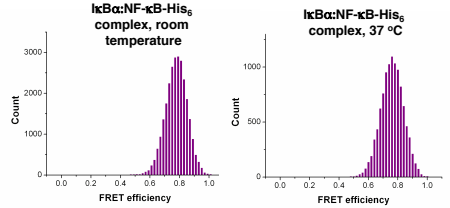
The experiments investigated the FRET efficiencies of Alexa-labeled **AR 2-6** in the presence and absence of NF-κB, at room temperature and 37 °C.



Sample traces of AR 2-6 molecules

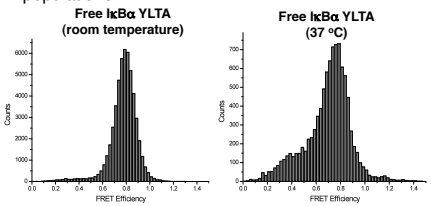


Swapping the His-tag: immobilization of AR 2-6 via His_6-tagged NF-κB



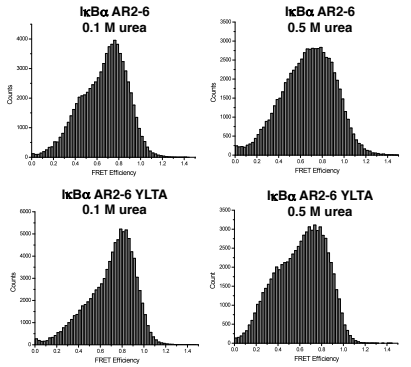
Pre-folding IκBα suppresses low-mid FRET

Introducing the Y254L/T257A mutations in repeat **6** (thereby constituting the ankyrin consensus sequence) results in reduced low-mid FRET populations.



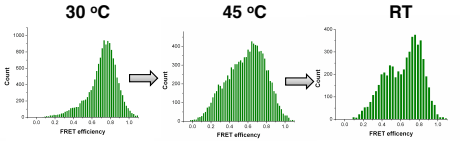
Urea denaturation experiments

Incubation of IκBα **AR 2-6** and **YLTA** constructs at low urea concentrations further unfolds the weakly folded repeats.



Thermal denaturation experiments

IκBα AR 2-6 becomes irreversibly denatured at 45 °C.



Future Directions

1. Proteasome degradation, temperature jump, urea flow-in experiments.
2. IκBα folding *in vivo*.

Acknowledgments

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