IκBα Structure and Dynamics Explored by FRET

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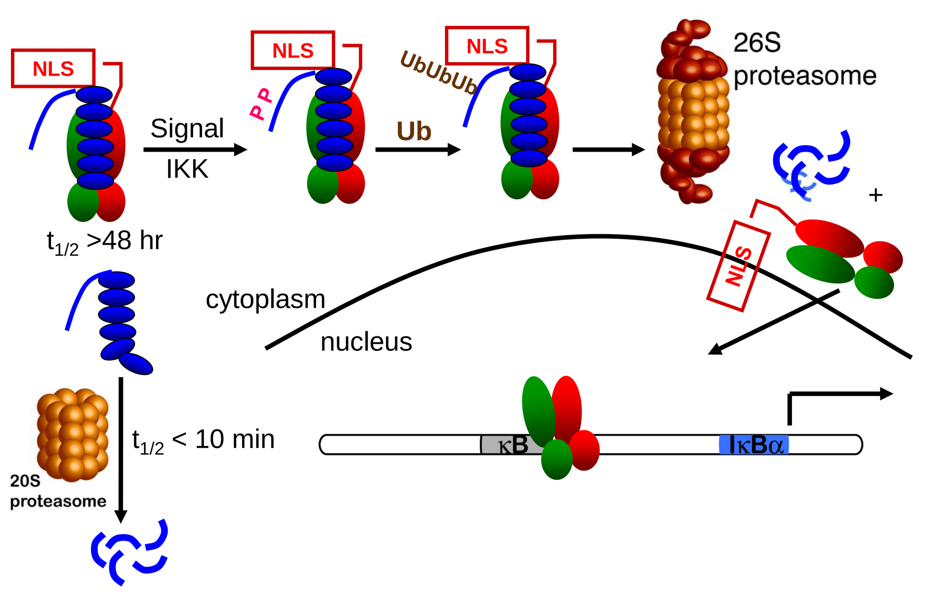
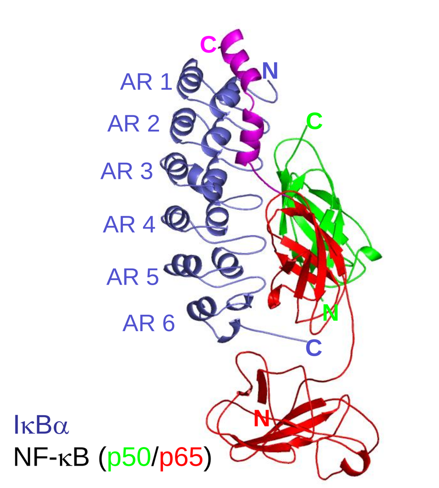
Introduction

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.

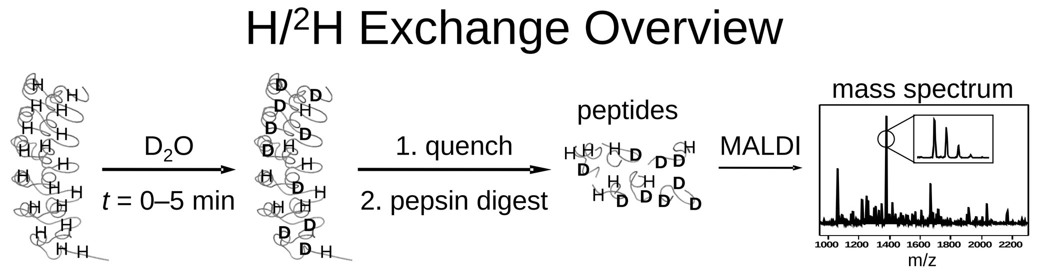
Crystal structure of IκBα provides only a static view when bound to NF-κB

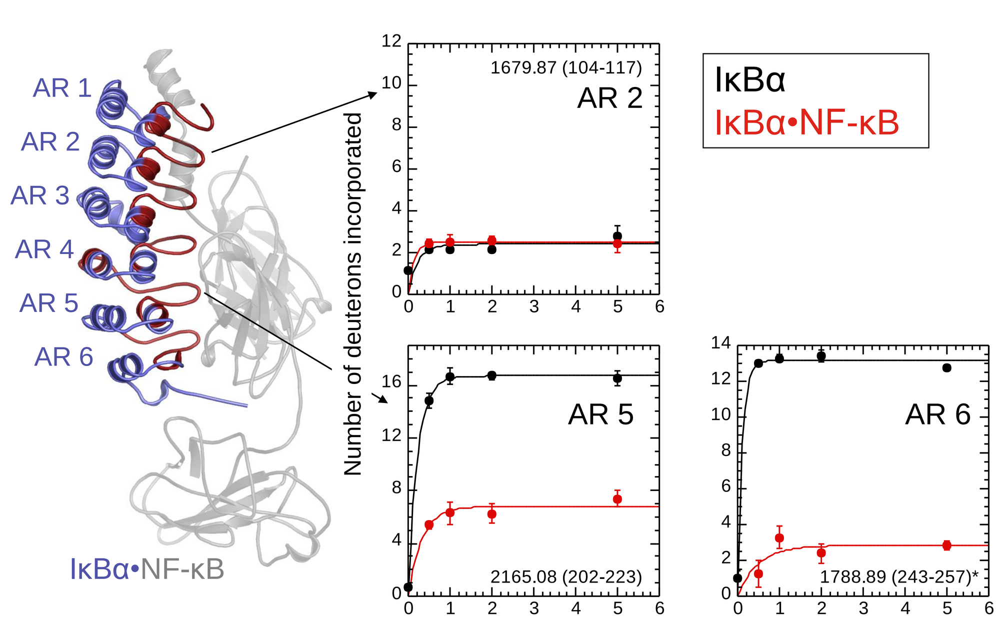


The crystal structure of IκBα (blue) bound to NF-κB (p50, green and p65, red; p65 NLS and flanking helices are magenta). IκBα contains six ankyrin repeats (AR), a ~33-amino-acid structural motif that generally adopts a helix-loop-helix-β-hairpin/loop fold. The p65 NLS and flanking helices (magenta) interact 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

H/^{2}H Exchange Overview



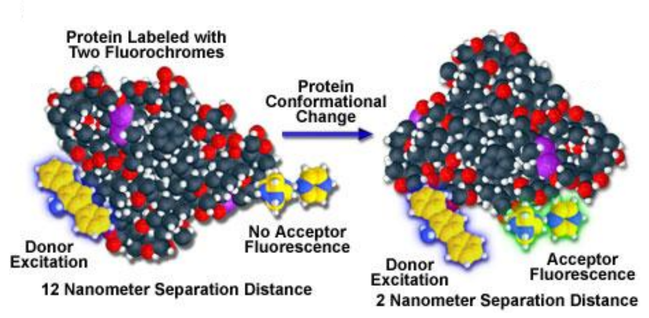


Truhlar, S. M.; Torpey, J. W.; Komives, E. A. *PNAS* **2006**, *103*, 18951-6.

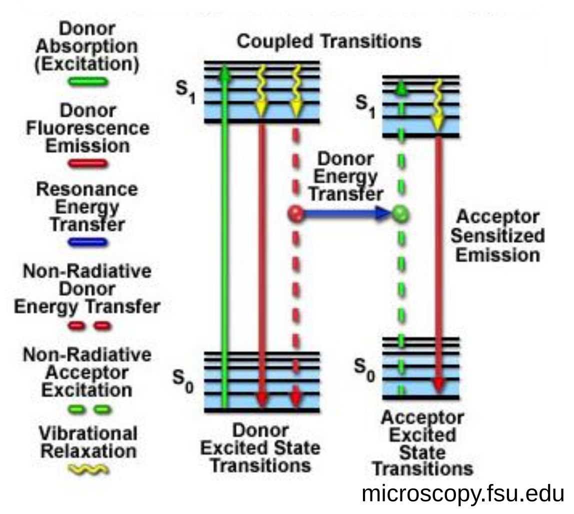
Förster Resonance Energy Transfer (FRET) as a tool for studying protein dynamics

Transfer of energy from a photo-excited donor to an acceptor fluorescent molecule located in close proximity.

Intramolecular FRET

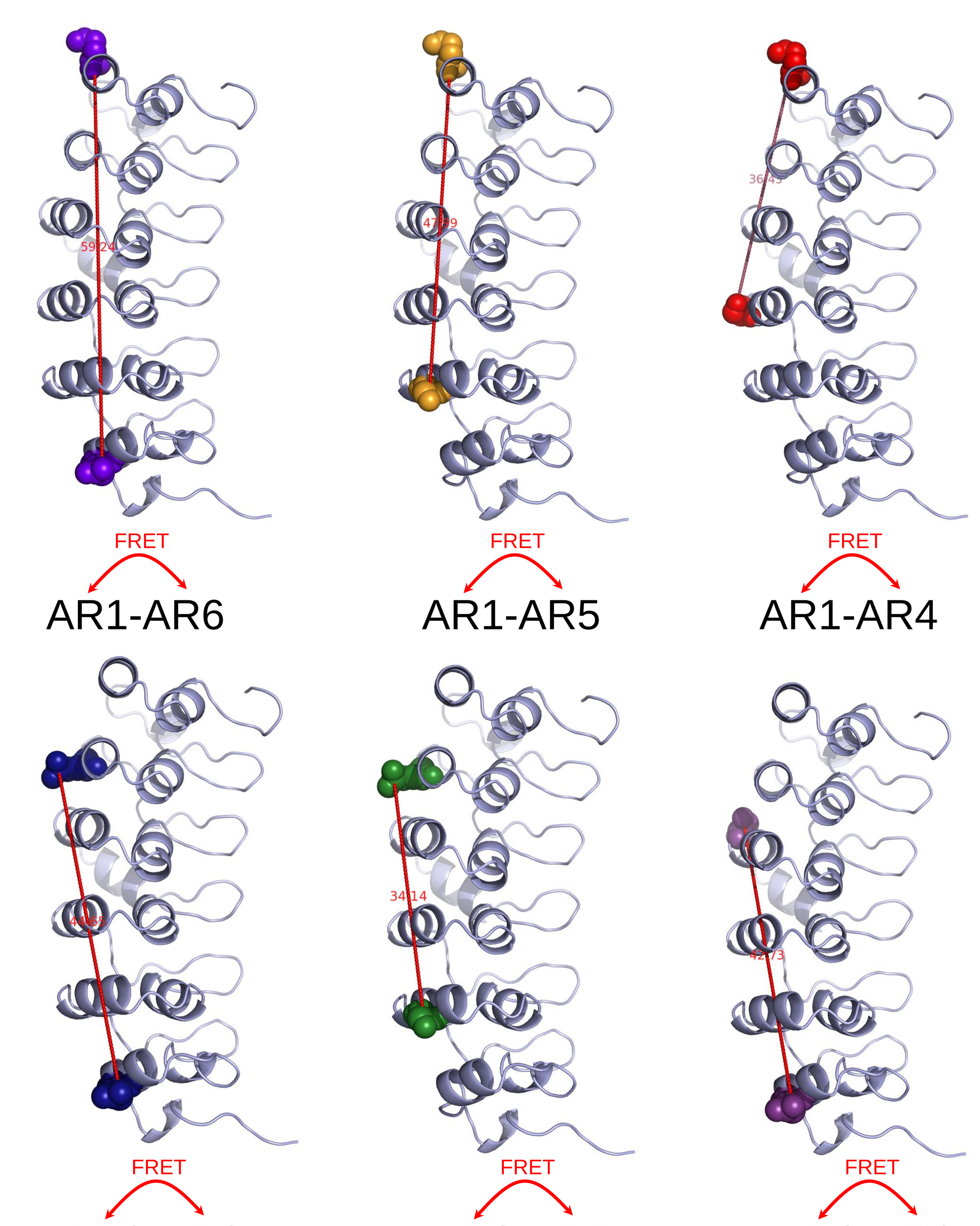


Resonance Energy Transfer Jablonski Diagram



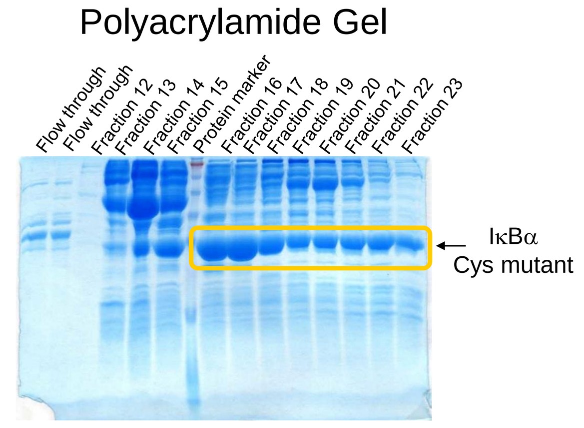
Engineered IκBα sites for conjugation of FRET fluorophores

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

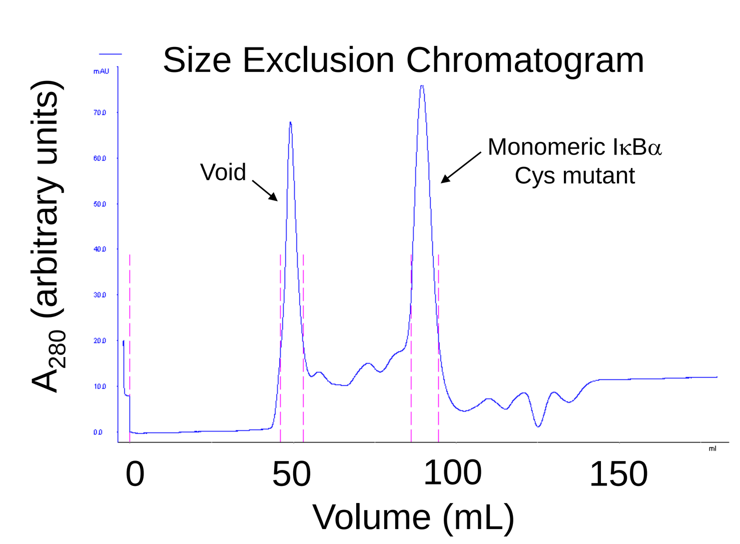


The IκBα cysteine mutants express well and remain monomeric

Polyacrylamide Gel

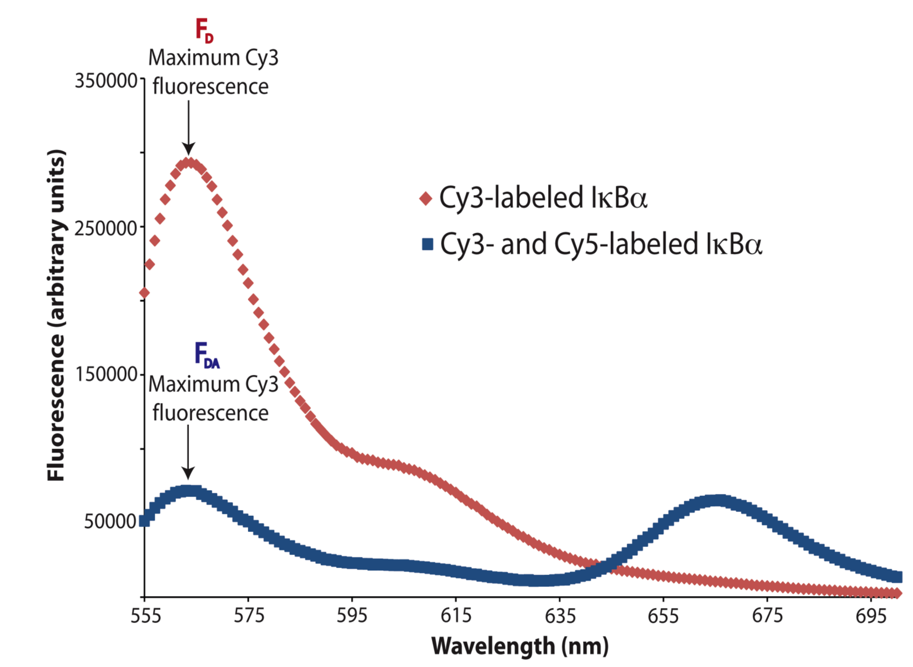


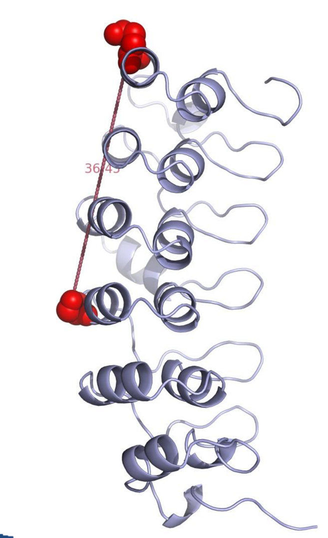
Size Exclusion Chromatogram



I. Bulk FRET Measurements

The experiments compared the FRET efficiencies of IκBα samples labeled with a single Cy3 fluorophore or Cy3/Cy5 pairs.





AR1-AR4 IκBα

Sample calculation of FRET efficiencies and inter-dye distances

I. Efficiency (E) calculation

$$E = 1 - (F_{DA}/F_D)$$

$$E = 0.76$$

II. Distance (r) calculation

$$r = \sqrt[6]{\frac{R_0^6 - (E * R_0^6)}{E}}$$

Using $R_0 = 60\text{Å}$

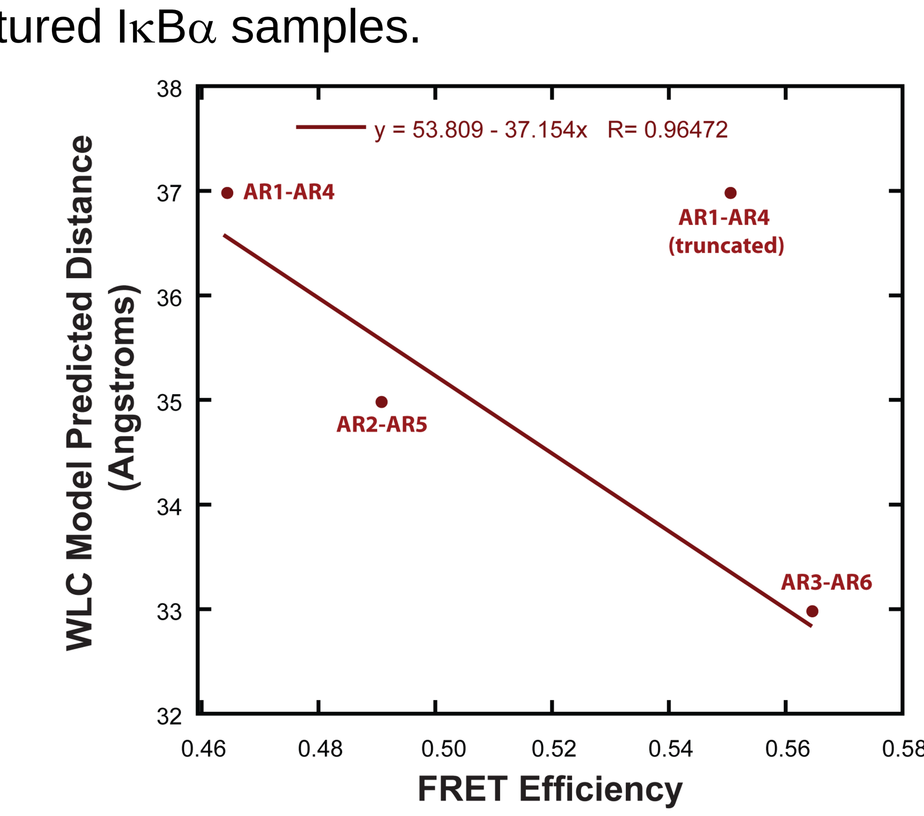
$$r = 50\text{Å}$$

Summary of FRET efficiencies and inter-dye distances

	Cy dyes		Alexa dyes	
Sample	*E*	*r* (Å)	*E*	*r* (Å)
AR1-AR4	0.76	50	0.49	54
AR2-AR5	--	--	0.42	57
AR3-AR6	0.48	61	0.32	61

Control experiment: FRET efficiency vs. worm-like chain (WLC) prediction distance

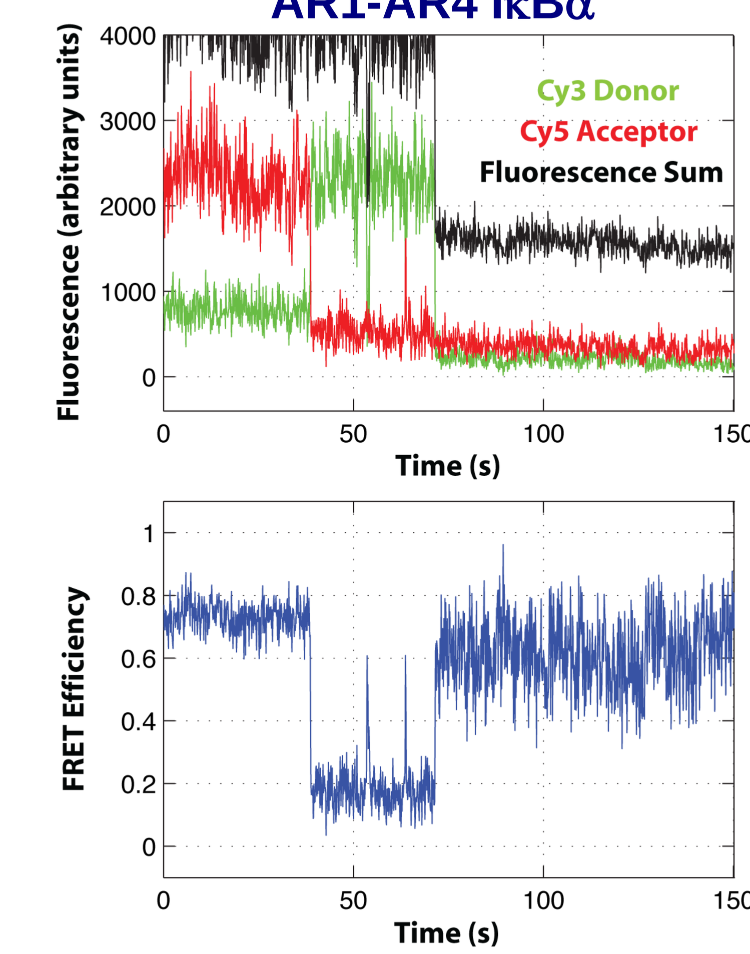
A linear relationship is observed between the predicted WLC distances and the FRET efficiencies of urea-denatured IκBα samples.



II. Single-Molecule FRET Measurements

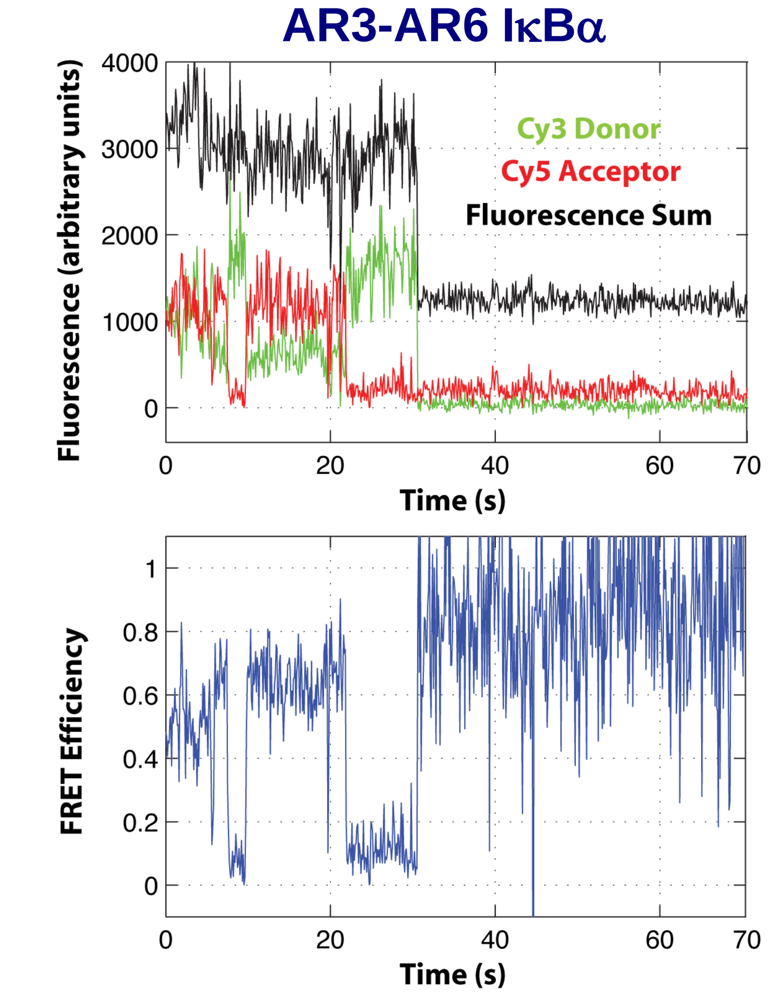
Single molecule experiments eliminate the signal averaging observed in bulk measurements and allows the direct observation of unique IκBα folding states.

AR1-AR4 IκBα



Constant FRET observed for the well-folded AR1-AR4 IκBα before Cy5 photobleaching occurs.

AR3-AR6 IκBα



Variable FRET observed for the weakly-folded AR3-AR6 IκBα. Switching of the FRET efficiency reflects the dynamic behavior of the sixth ankyrin repeat.

Future Directions

1. Investigate IκBα folding dynamics in the presence of NF-κB using bulk and single-molecule FRET.
2. Measure bulk and single-molecule FRET of stable, pre-folded IκBα mutants.
3. Use an AcGFP1-IκBα-mCherry fusion protein to investigate IκBα dynamics *in vivo*, in the presence and absence of NF-κB.

Acknowledgments

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