Use of FRET for detecting conformational changes in proteins and molecular binding in cells

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Fluorescence resonance energy transfer spectroscopy (or FRET) has long been used to monitor molecular distances, particularly between proteins, but also between nucleotides. The basic requirement of the technique is to place a fluorescent donor at a specified but unique place on a protein, and place an acceptor (which may or may not be fluorescent) at another specific location, either on the same molecule or on a ligand such as another protein. Depending on the spectral properties of the donor and acceptor, distances ranging from 1.0 through to about 10.0 nm can be measured. In practise, the determination of these molecular distance are not highly precise but it is accurate, but it can be highly precise. Thus, FRET is particularly good at monitoring changes in molecular distances. Thus, FRET is probably best used to detect small structural changes such as changes in protein conformation rather than actual molecular distances.

Actin is the principal component of microfilaments whose assembly/disassembly is essential for cell motility. This assembly is regulated by a number of actin-binding proteins (ABPs), particularly cofilin and thymosin β_4. We have published several papers where we detect conformational changes in actin when ABPs bind. In 2003 we reported that different ABPs (cofilin and DNase I) can have opposite structural effects on the conformation of actin, and this month we reported that thymosin β_4 (Biophysical Journal) binding induces spatial rearrangements within the small domain (subdomains 1 and 2) of actin monomers.

Actin and cofilin are also present in the nucleus where actin probably plays a role in regulating the activity of DNase I, particularly when the cell is stressed. Cofilin can bind actin and translocate it into the nucleus during times of stress. We combined FRET and confocal microscopy to analyze the interactions of cofilin and G-actin in the nucleus and cytoplasm. By measuring the rate of photobleaching of fluorescein-labeled actin ± Cy5-labeled cofilin, we show that most of the nuclear G-actin is bound to cofilin, but only half is bound in the cytoplasm. A significant proportion of cofilin in the nucleus and cytoplasm binds added TMR-labeled G-actin. These data suggest there is significantly more cofilin-G-actin complex in the nucleus and less free cofilin in the nucleus.

These examples of FRET show how it can be used to monitor molecular changes when proteins bind *in vitro*, but it can also be used in cells to distinguish between proteins that lie in the same confocal volume but are not bound and those that are bound.