Figure 1 Romoser *et al.*⁶, Persechini *et al.*⁷ and Miyawaki *et al.*⁸ have linked blue-shifted GFP mutants to longer-wavelength GFP mutants through connecting sequences to which Ca^{2+} -calmodulin⁶ or Ca^{2+} alone^{7,8} can bind. The two GFPs are X-ray structures from J. Remington; the calmodulin-binding domain is the NMR structure from M. Ikura.

These chimaeric proteins can be used as Ca^{2+} indicators because a change in the concentration of Ca^{2+} results in a change in the ratio between the intensity of light that is emitted at two wavelengths — 510/440 nm in the studies by Persechini and colleagues^{6,7}, and 535/480 nm in the paper by Miyawaki *et al.*⁸. The short calmodulin-binding peptide used by Romoser *et al.*⁶ holds the two flanking GFPs at an optimal distance for efficient FRET in the absence of Ca^{2+} . In the work by Persechini *et al.*⁷, the amino- and carboxy-terminal EF hand domains of calmodulin have been introduced into the construct. Binding of unlabelled Ca^{2+} -calmodulin (or Ca^{2+} in ref. 7) straightens the linker peptide and decreases FRET.

In Miyawaki and colleagues' *cameleon* constructs, the linker between the two GFPs is calmodulin, fused to a calmodulin-binding peptide (M13). On binding Ca^{2+} , the calmodulin wraps around its adjacent target peptide, bringing the two GFPs closer to each other and increasing FRET. By building the calmodulin into the indicator⁸ or including calmodulin Ca^{2+} -binding domains⁷, the expression levels of the calmodulin and its target peptide are guaranteed to be equal. Moreover, the affinity and kinetics of Ca^{2+} -binding will be independent of the concentration of indicator; the likelihood of disrupting endogenous calmodulin-dependent signalling is reduced; and mutation of the calmodulin can tune the Ca^{2+} -sensitivity, to cover free Ca^{2+} from nanomolar to millimolar concentrations.

With the help of humanized codons and mutations to increase the level of expression and folding, the indicators can be introduced into cells by transfecting the gene⁸ rather than by microinjecting the protein^{6,7}. Transfection allows targeting sequences to be included, which send the indicator to specific compartments such as the nucleoplasm and the lumen of the endoplasmic reticulum. Targeted *cameleons* already seem to be superior to the other tools that are available for monitoring the dynamics of Ca^{2+} in such compartments. For example, although the Ca^{2+} -sensitive photoprotein aequorin can be targeted to cellular organelles with similar selectivity¹⁰, it requires the coenzyme coelenterazine, and its Ca^{2+} -dependent chemiluminescent signal is less amenable to single-cell imaging.

From the point of view of cellular Ca^{2+} homeostasis, the most important observation made by Miyawaki *et al.*⁸ is that the concentration of Ca^{2+} within the endoplasmic reticulum at steady state — an issue that has been widely debated during the past few years — is two to three orders of magnitude higher than in the cytosol. The authors report that the concentration of Ca^{2+} in their cell population ranged from around 400 μM to about 50 μM . The higher values were found in most cells, and were close to those measured with targeted aequorin and synthetic coelenterazine¹¹. The lower values were found in only about 10 per cent of cells (A. Miyawaki and J. Llopis, personal communication).

A final, highly interesting observation deserves a mention. One of Miyawaki and colleagues' constructs can monitor intermolecular rather than intramolecular FRET. From a general point of view, this result is the most exciting. Many groups, including mine, are pursuing the possibility of studying protein-protein interactions in living cells, using different protein partners tagged with GFP mutants and FRET to monitor their reversible association. To my knowledge, this is the first demonstration that a dynamically modulated protein-protein interaction can be followed using molecularly engineered GFP fusion proteins in live cells. Given the vast number of key cell functions that are governed by association between polypeptide partners, it is easy to predict that the new findings will induce many new laboratories to undertake this approach. □

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Daedalus

A biased solid

Woven cloth is the oldest fibre-reinforced material. It is merely held together by the friction of the fibres threaded past each other, yet is amazingly durable. Furthermore, it has the useful property of being extremely weak in shear. A square of cloth can easily be pulled sideways into a parallelogram; instead of crossing each other at right angles, the warp and weft fibres merely do so at a slightly different angle. In couture, this property is neatly exploited in the 'bias cut', invented by Mme Vionnet in 1922. In a bias-cut garment the fibres run, not vertically and horizontally, but at 45°. It can stretch vertically, while shrinking horizontally to emphasize the wearer's figure.

Daedalus is now extending this idea to three dimensions. Just as two sets of fibres can be interwoven at right angles to make a flat cloth, so three sets at right angles could create a fibrous solid. A three-dimensional loom is as simple in principle, though more complicated in practice, than the normal planar variety. Daedalus's design has a square array of parallel threads running vertically, and two sets of shuttles at right angles running horizontally through the array. As each layer of interwoven horizontal threads is complete, beaters pack it down on the previous layers. The result is a solid block of three interwoven orthogonal sets of fibres.

This novel product will have many uses. Its toughness and tenacity, its ability to give slightly in any direction without breaking or even weakening, will make it ideal for all sorts of buffers, seals, shock-absorbers and load-spreaders. But Daedalus wants to cut it on the bias. The resulting 'Vionnet solid' would have enormous tensile strength, but very low shear strength. Push any two sides together, and they will come; but the other sides will extend to maintain its constant volume.

A shaped Vionnet solid will be a wonderfully forgiving engineering component. If it does not fit in the hole made for it, just stretch it till it does; once it is in place, just push it in and it will swell to grip its surroundings. In cotton, polyester or even metal wire, Vionnet blocks and forms will bring a new flexibility to construction and engineering. They will forgive amateurs their mistakes and relax the worries of professionals. Furthermore, when finally in place, they could be soaked in polymer resin, when they would harden into the first truly isotropic fibre-reinforced composite.

David Jones