Looking at Proteins Interacting and Folding inside Cells

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Fluorescence microscopy has revolutionized cell biology. Not only has it increased the spatial resolution with which certain processes are visualized, but it has allowed microscopists to monitor more than one event at a time. One of the remaining challenges is to see how proteins fold and assume their native conformations within living cells. Recently, Nathan Luedtke, Rachel Dexter, Daniel Fried, and Alanna Schepartz have demonstrated a technique to demonstrate these events *in situ*.[2]

Luedtke *et al.* pointed out that Förster resonance energy transfer (FRET) techniques are limited in resolution by normal variations in cell-to-cell fluorescence intensity. In general, energy intensity variations below 50% are difficult to resolve from normal variations. Also the size of large fluorescent tags, such as green fluorescence protein, can increase the steric bulk of tagged proteins and influence their kinetics. They proposed an alternative strategy using "profluorescent" biarsenical dyes called FlasH-EDT$_2$ and ReAsH-EDT$_2$. These small molecules can enter cells and selectively label recombinant proteins that contain a tag with the amino acid sequence of cysteine-cysteine-proline-glycine-cysteine-cysteine (CCPGCC). The 1,2-ethanedithiol (EDT) part of the dye is changed within the cell, leading to the formation of a highly fluorescent protein-bound complex. Tetracysteine incorporation and subsequent biarsenical labeling was not as disruptive to protein functions as other fluorescent tags. For several reasons, Luedtke *et al.* thought that the CCPGCC motif could function as a reporter of protein conformation or certain protein-protein interactions where the two pairs of cysteine molecules would be brought in close proximity to each other (about 7Å) and then bind to FlAsH or ReAsH to form a fluorescent complex.

Luedtke *et al.* performed several experiments *in vitro* that demonstrated well-folded polypeptides and protein-protein domains, when appropriately modified so that the cysteine molecules were aligned, bind biarsenical dyes with high affinity and can form complexes with fluorescence intensities that approximated the optimized tetracysteine motif. They also showed that FlAsH complexed to proteins that folded in a nearly natural manner and had the expected stoichiometries. Furthermore, FlAsH and ReAsH could be used to distinguish properly folded from misfolded polypeptides.

Next Luedtke *et al.* demonstrated that FlAsH and ReAsH could detect protein assembly and folding in live mammalian cells. The differences in fluorescent brightness between cells expressing wild-type and misfolded polypeptides were clearly visible by microscopy in real time without using multicolor imagining or false coloration methods. They also correlated ReAsH fluorescence with other methods to account for cell-to-cell variations and found ReAsH to be much more sensitive. They concluded that this technique can be used to detect discrete conformational states and stable protein-protein interactions in living cells, even to the extent of distinguishing proteins that differ by a single amino acid.

The value of this newly-developed fluorescent technique will be interesting to observe in future studies of basic cell biology. Luedtke *et al.* also pointed out that it will be valuable in unraveling pathologic mechanisms. Many human diseases have protein misfolding and aggregation as their core cause; sickle cell anemia, Alzheimer disease, and Parkinson disease being a few examples. The extreme proximity of cysteine molecules required for this technique promises greater spatial resolution than possible with other methods. Finally, this technique has the potential to be combined with other advanced fluorescent applications, possibly enabling even higher resolution imaging and/or inactivation of discrete protein complexes. In turn, this could lead to high-throughput screening for detecting early protein misfolding, providing an important novel diagnostic tool.

1 The author gratefully acknowledges Dr. Alanna Schepartz for reviewing this article.

2 Luedtke, N.W., R.J. Dexter, D.B. Fried, and A. Schepartz, Surveying polypeptide and protein domain configuration and association with FlAsH and ReAsH, *Nature Chem. Biol.* **3**:779-784, 2007.

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ABOUT THE COVER

The cover shows the ice researchers field camp located on the shore of Lake Bonney, at the terminus of Taylor Glacier in Antarctica. The experimental site was located 2km up valley from the camp and is, in turn, located 120km from McMurdo Sound Station, the largest settlement on the continent. Please see the article by Doyle, Amato and Christner beginning on page 6.

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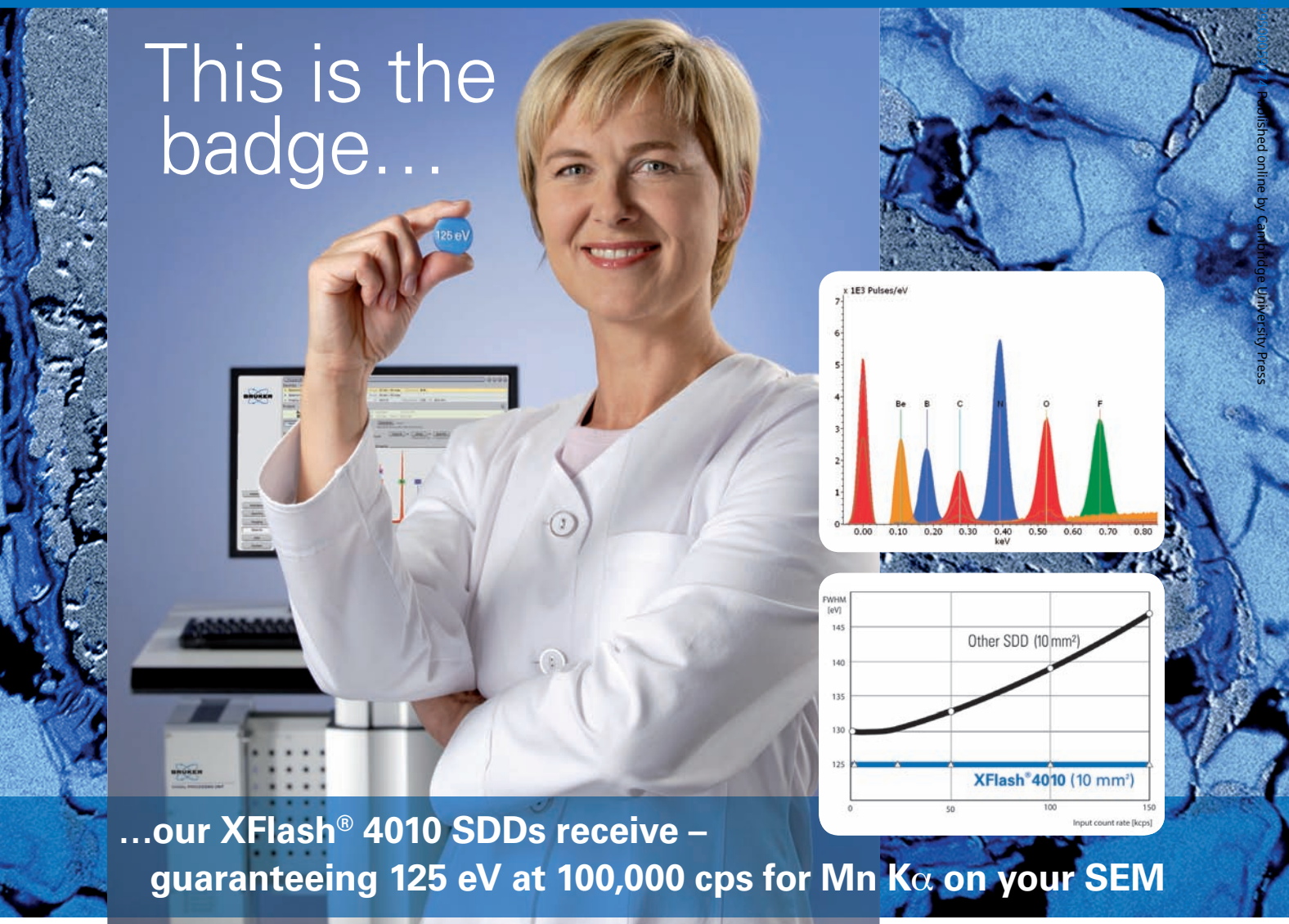

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