Semiconductor nanocrystal conjugates, FISH and pH

To the editor: Properties of high luminescence, reduced photobleaching and single-wavelength excitation make quantum dots (QDs) ideal for bioimaging¹. Fluorescence *in situ* hybridization (FISH) applications have been limited for lack of reagents, stable surface treatments² and optimization. We previously demonstrated QD-FISH detection of DNA probes with (CdSe)/ZnS-streptavidin conjugates³. Here we show pH effects on fluorescence of QD-detected hybridization signals in FISH experiments that are not evident in solution experiments.

FISH (Fig. 1a) revealed major hybridization sites irrespective of the type of conjugate (organic or nanocrystal). We were able to detect the centromeres of other human chromosomes with organic fluorophores, but not with quantum dots (Fig. 1a).

We investigated the effects of pH on fluorescence with biotinylated total human genomic DNA probes in FISH experiments. At each pH, cell manipulations were identical before the final incubation

with fluorophore-streptavidin conjugates. Fluorescence intensities of chromosome band 1q12 (ref. 4) and total chromosomal signal were plotted (Fig. 1b). QD fluorophore intensities varied with pH in two different imaging systems (Fig. 1b,c). QD signals were optimal at ~pH 6–7, and diminished at pH 4 and pH 8 (Fig. 1b, manually quantified FISH). Standard deviations at pH 6 and pH 7 overlapped (Fig. 1b). Laser scanning cytometry (LSC) experiments confirmed this finding (5,000–10,000 cells at each pH; Fig. 1c). LSC measurements included the number of signals in each cell and the integrated density of all QD signals (Fig. 1c). These data confirmed that borate buffers at pH 6 and 7 gave superior fluorescence intensity. Quantification showed fluorescence intensity was greater at pH 6 than pH 7 by twofold (Fig. 1c). Previously, we³ noted qualitative distribution differences of fluorescence sites between fluorescein isothiocyanate (FITC) and QD detection systems. Here we quantitatively show that spot number at pH 6 was greater than at lower or higher pH (Fig. 1c). Rescanning FISH/LSC experiments demonstrated that QD fluorescence was more stable (98% after five scans) than Alexa Fluor 488 (75% after five scans), extending this analysis to high-throughput imaging (Supplementary Fig. 1 online). Single-cell FISH with dynamic changes in pH via microfluidics using a third imaging system also confirmed this (Supplementary Data and Supplementary Fig. 2 online). Thus, fluorescence intensity of streptavidin-QD conjugates used to detect FISH hybridization probes varied with pH of the final incubation buffer.

Control solution experiments with QD conjugates in the presence or absence of DNA showed no pH dependence (Supplementary Data). It is unclear why fluorescence intensity of the QD-streptavidin probe in the FISH format exhibited pH dependence, pH control, however, may allow optimization of QD fluorophores in clinically useful formats such as FISH and other hybridization-based assays.

Additional information is available in **Supplementary Methods** online.

Note: Supplementary information is available on the Nature Methods website.

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1. Alivisatos, P. *Nat. Biotechnol.* **22**, 47–52 (2004).
2. Pathak, S., Choi, S.-K., Arnheim, N. & Thompson, M.E. *J. Am. Chem. Soc.* **123**, 4103–4104 (2001).
3. Xiao, Y. & Barker, P.E. *Nucleic Acids Res.* **32**, e28 (2004).

Figure 1 | pH dependence of fluorescence intensity in FISH experiments with total genomic DNA detected by Texas red and QDs. (a) Distribution of FISH signals detected with Texas red-streptavidin conjugate (top); and distribution of Qdot 605-streptavidin detected FISH signals (bottom). Images from three different experiments are shown to demonstrate the consistency of the labeling difference. Bar, 10 μ m. (b) Signal intensity digital quantitation of FISH signal at chromosome band 1q12 or total signal ($n = 3$ cells, $\pm$ s.d.). In a and b, fluorescence intensity was measured by digital imaging with mercury lamp illumination. (c) Identical FISH experiment quantitated by LSC. Left, total number of spots in each cell; right, intensity of total FISH signal. ($n = 5,000$ –10,000 cells with median value, left or integral, right).