Application of highly luminescent quantum dot bioconjugates in protein imaging: quantum dot-based immunoblot analysis

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The highly luminescent semiconductor quantum dots (QDots) have attracted a great interest for life science research, because of their potential to be used as new fluorescent probes in cell and protein imaging techniques. The interest to QDots is based on their higher brightness than the conventional fluorophores and privilege for single-source excitation for all colors.

The present study describes a synthesis of highly luminescent Qdot-bioconjugates and development of method for Qdot-based Western blot analysis. Water-soluble CdSe quantum dots (~2-3 nm) were conjugated with several antibodies - anti-c-abl, anti-lamin A/C and anti-β-actin, and were applied for immunoblot analysis of the respective proteins in leukemia cells (K-562 - derived from chronic myelogenous leukemia, and Jurkat – derived from acute lymphoblastic leukemia). In Qdot-based Western blot analysis we used only a primary antibody and the photoluminescence of Qdot-antibody conjugates, retained on PVDF-membrane, was detected by ChemiImager. The described procedure avoided the application of secondary antibody and subsequent HRP-catalyzed enzyme reaction with formation of luminescent product, which often compromises the results after ensuring saturation. The sensitivity of Qdot-based immunoblot analysis is about 3 times higher than that of the conventional Western blot procedure. However, the photobleaching of Qdot-labeled blots was faster and the stability of Qdot-antibody blotted membranes was lower than in conventional immunoblot procedure. To ensure higher efficiency and to guarantee comparatively high stability of Qdot-blot photoluminescence, biotinilated antibodies were covalently conjugated with Qdot and sandwich-type avidin-biotin assay system was additionally applied, using Qdot-avidin and Qdot-biotin conjugates.