SUPPORTING INFORMATION PARAGRAPH

Reagents: Organic dyes (FITC- and Alexa Fluor 647-Annexin V conjugates) were purchased from Molecular Probes (Invitrogen, Breda, The Netherlands). Qdots 585 Streptavidin Conjugates were purchased from Quantum Dot Corporation (Tebu-bio bv, Heerhugowaard, The Netherlands). Chemicals (etoposide, cycloheximide, TNF alpha, camptothecin, Annexin V-biotin) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands).

Cell culture and apoptosis induction: Human promyeloctic leukemic HL60 cells were obtained from the German Collection of Microorganisms (Braunschweig, Germany). Tissue culture equipment was purchased from Greiner bio-one (Frickenhausen, Germany). HL 60 cells were cultured in RPMI-1640 medium (Gibco, Invitrogen) supplemented with 10 % heat-inactivated and filter-sterilized Foetal Calf Serum, 100 IU/ml penicillin, 100 mg/mL streptomycin, 2 mM L-Glutamine and 250 μg/mL fungizone ($RPMI^+$ medium). Supplements and antibiotics were purchased from (Invitrogen). Cell cultures were maintained at 37ºC and in a humidified environment with 5% CO_2. Medium was changed every 4-5 days. Cell concentration was assessed to be of approximately 10^6 cell/mL. Experiments were carried out on exponentially growing cells.

Apoptosis was induced by incubation of cells under culturing conditions with drugs or upon UV irradiation. Several apoptosis inducing means were tested here, etoposide at 50 μM, TNF-alpha at 3 nM and cycloheximide at 50 μM, camptothecin at 6 μM and UV irradiation (λ = 254 nm, I = 2×6 Watt) for 5 minutes. After irradiation or the addition of drugs, cells were placed back overnight in the incubator (37ºC, 5% CO_2).

Staining of cells: Cells were stained with organic dyes and quantum dots, both targeted at phosphatidylserine (PS) recognition. Qdots staining was achieved through two ways, either with a consecutive binding on cells of biotinylated Annexin V and Qdots Streptavidin Conjugates or with a separate preparation of Qdots-Annexin V, subsequently added to cells. Control experiments were also carried out on healthy cells (without induction of apoptosis).

Separate preparation of Qdots-Annexin V conjugates: Annexin V-conjugated quantum dots were first and separately prepared. 2 μL of Qdots (2 pmol) were dissolved in 10 μL of PBS 1× buffer; this solution was then vortexed for 30 s and added to 10 μL of PBS 1×buffer containing biotinylated Annexin V (1 μL; ~ 4 pmol). The resulting mixture was allowed to react for approximately one hour at room temperature, and added to cells (500 μL at 10^6 cells/mL) suspended in a $CaCl_2$-containing medium (2.5 mM). Incubation with Qdots was carried out at 37ºC for typically 60 min. Counterstaining was simultaneously performed by adding an Annexin V-conjugated organic dye, such as Alexa Fluor 647- or FITC-conjugated Annexin V (0.5 % v/v). Cells were finally washed, resuspended in fresh phenol red-free medium before imaging and placed in appropriate chamber plates (Nalge Nunc, VWR, Roden, The Netherlands) for confocal fluorescence microscopy.

Consecutive binding: Apoptotic cells were pre-incubated with Annexin V. Typically, cells (500 μL at 10^6 cells/mL) were resuspended in a $CaCl_2$ containing buffer (2.5 mM) to which an apoptosis inducer (e.g. etoposide at 50 μM) as well as biotinylated Annexin V (0.5 μL, ~2 pmol) were added, and let overnight for incubation (37ºC, 5% CO_2). Following this, cells were incubated with Qdots (2 μL, 2 pmol) preliminarily

dissolved in 20 μL of PBS 1×buffer for 60 min at 37ºC. Finally, a FITC- or an Alexa Fluor 647-Annexin V conjugate was added (0.5 % v/v). After approximately 30 min of incubation (37ºC, 5 % CO_2), cells were washed, resuspended in fresh phenol-red free medium, and imaged.

Confocal microscopy: Confocal laser scanning microscopy was performed with a Zeiss LSM 510-meta system using a 63X oil immersion objective. Excitation and filters were as follows: FITC, 488 nm excitation, emission BP 500-500 nm filter; Alexa Fluor 647, 633 nm excitation, LP 650 nm filter; Qdots, 543 nm excitation, LP 560 nm filter. A multi-track configuration was used when several dyes were simultaneously used; this may results in a slight shift between the various colored views. It should be noted that care was taken to decrease the laser intensity as much as possible to limit cell injury and bleaching of organic dyes. Laser intensity was fixed at 5 % and 10 % for FITC and Alexa Fluor 647 respectively.