We have generated a growing family of cationic dyes with interesting fluorescent properties that are markedly environment sensitive (e.g., DNA, protein, heparin or artificial membranes). Our library of 1500 dyes is based on 19 skeletal structures which are easily modified by standard commercially available chemicals to achieve desired properties. In contrast to a majority of commercial organic dyes our dyes are nonplanar, a feature which may bring new opportunities for detection and visualization of important biological structures. Altogether, this indicates their potential use in various applications such as flow cytometry and microscopy.

A set of 150 selected dyes have been tested for their ability to penetrate and label specific structures in human cell lines (HeLa, CCRF-CEM, HGC-27, Hep G2 and U2-OS). Highly selective labelling of mitochondria was prevalent for our novel dyes but other localizations such as plasma membrane, endoplasmic reticulum or nucleolus can be detected. A good example of specificity of our novel fluorescent probes are dyes Cellmem8 and Mito19 which were shown to highly colocalise with commercial dyes CellMask™ (PCC = 0.73) and MitoTracker® (PCC = 0.81), respectively, see Figures 1 and 2. Conversely, DNA sensitive dye Celldead3 cannot penetrate cytoplasmatic membrane and therefore is able to discriminate dead cells, see Figure 3.

Our dyes show good biocompatibility and are promising candidates for live-cell imaging. The simple one step synthesis and easy purification process make them even more attractive.

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Fig. 1: Colocalization of Cellmem8 (A) with commercial dye CellMask™ Deep Red (B) shown in HeLa cells. C shows merge of the images A and B. Zeiss LSM 780, Plan-Apo 63x / 1.4 NA oil immersion objective.

Fig. 2: Colocalization of Mito19 (A) with commercial dye MitoTracker® Red (B) shown in HeLa cells. C shows merge of the images A and B. Zeiss LSM 780, Plan-Apo 63x / 1.4 NA oil immersion objective.

Fig. 3: Staining of dead cells (HGC-27) with our dye – Celldead3 and GelRed. A shows the merge of bright field and cells stained with Celldead3 and GelRed. B shows the colocalization (PCC = 0.83) of Celldead3 (C) and GelRed (D). Zeiss AxioObserver.A1, 20x objective.