

Spectroscopic and Cytotoxicity Studies of a Family of Asymmetric Cyanine Dyes

J. Pauli¹, T. Vag², W. A. Kaiser², I. Hilger², U. Resch-Genger¹

¹Federal Institute for Materials Research and Testing (BAM), Richard-Willstätter-Str. 11, D-12489 Berlin

²Institute for Diagnostic and Interventional Radiology, Friedrich-Schiller-University Jena, Erlanger Allee 101, D-07747 Jena

Introduction

The applicability and sensitivity of in vivo near-infrared fluorescence (NIRF) imaging depends on the cytotoxicity and the spectroscopic properties of the fluorophores. Suitable dyes are characterized by low cytotoxicity, high stability and a high molar absorption coefficient as well as a high fluorescence quantum yield Φ_f under application-relevant conditions. The only moderate fluorescence quantum yields of DY-676-conjugated antibody fragments (FabAntiCEA-DY-676) recently exploited by us for selective monitoring of CEA expressing tumor cells in mice¹⁾ encouraged us to search for improved fluorophores, absorbing and emitting in the typical excitation (650 - 750nm) and detection ranges (650 - 950nm) of NIRF scanners.

Cytotoxicity

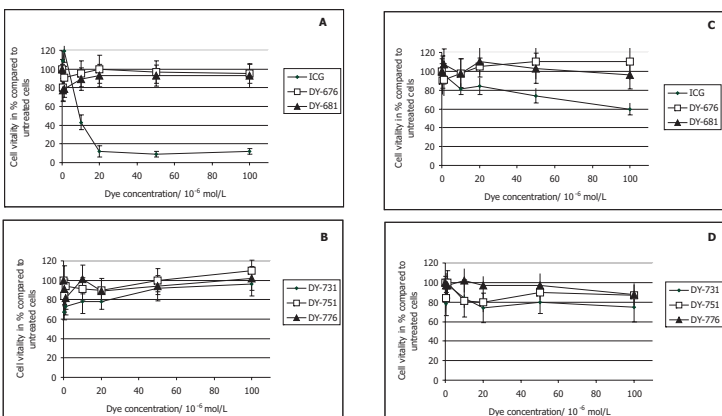


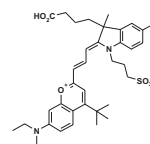
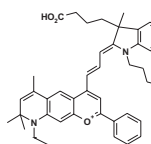
Fig. 2. Cytotoxicity of the DY dyes and ICG determined for a macrophage cell line (J774; panel A+B) and an endothelial cell line (SVEC4-10; panel C+D) after 72h of incubation with each dye. Cell cultures without any dye treatment exhibited a cell vitality of 100%.

Structures

Studied asymmetric cyanines (DYOMICS GmbH):

DY-676

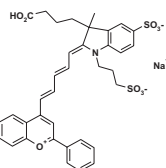
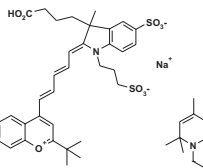
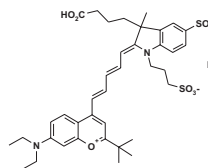
DY-681



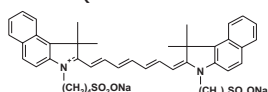
DY-731

DY-751

DY-776



Symmetric cyanine ICG (Pulsion Medical Systems):



Acknowledgement

We are grateful to DYOMICS GmbH (Jena, Germany) for the generous supply of the dyes DY-676, DY-681, DY-731, DY-751, and DY-776.

Quantum Yield Values Φ_f

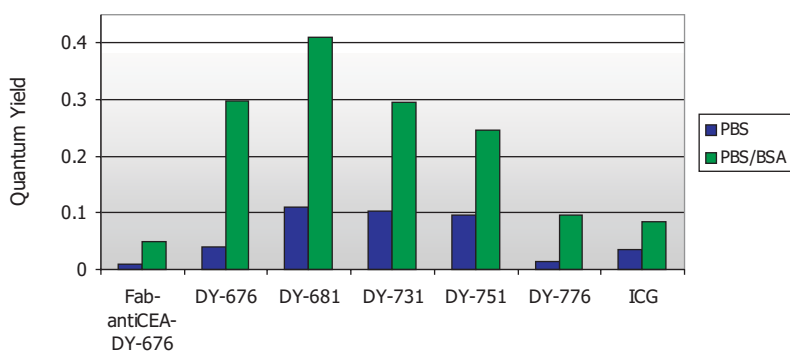


Fig. 1 Φ_f values of the DY dyes and ICG in PBS/BSA (green) and PBS (blue)

- ▶ The Φ_f values of the DY dyes in PBS/BSA are always higher than that of ICG.
- ▶ For each dye, Φ_f in PBS/BSA exceeds that of Φ_f in PBS.
- ▶ DY-681 has the highest Φ_f of 0.40 in PBS/BSA.
- ▶ Maximum Φ_f values in PBS of 0.10 were found for DY-731 and DY-751.
- ▶ For both cell types, the DY dyes displayed lower cytotoxic effects compared to ICG at a dye concentration between 20 and 100 μM .

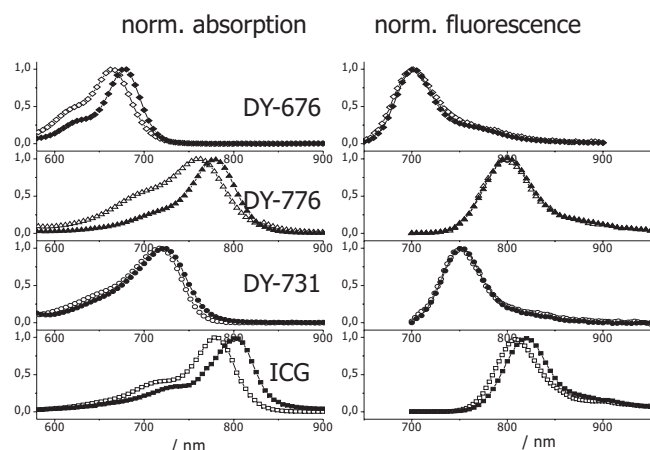


Fig. 3 Comparison of the absorption and fluorescence spectra in PBS/BSA (full signs) and in PBS (open signs).

- ▶ Spectral broadening in absorption suggests formation of nonfluorescent aggregates for DY-676 and DY-776 in PBS yielding low Φ_f .

Reference

- 1) M.-R. Lisy, A. Goermar, C. Thomas, J. Pauli, U. Resch-Genger, W. A. Kaiser, I. Hilger: In Vivo Near-Infrared Fluorescence Imaging of Carcinoembryonic Antigen-expressing Tumor Cells in Mice, *Radiology*, 2008, 247, 779-787
- 2) T. Vag, J. Pauli, R. Haag, M. Spieles, M. Wenzel, U. Resch-Genger, W. A. Kaiser, I. Hilger: New Near Infrared Dyes for Molecular Imaging. An In Vitro Characterization Study. *Eur J Med Chem*, submitted.