Atto647n-lysine-dextran

CAS nr: N/A

Structure:

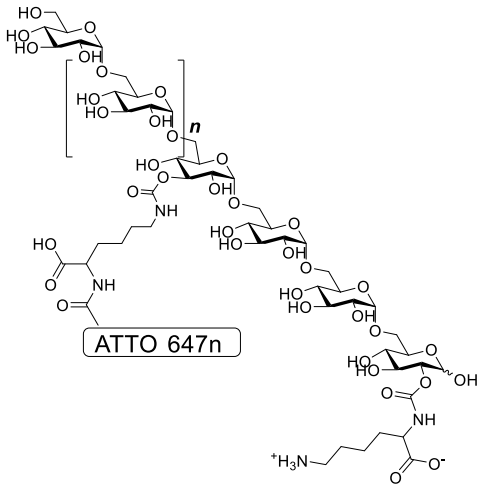


Fig. 1. Structural representation of Atto647n-lysine-dextran

Synthesis and structure

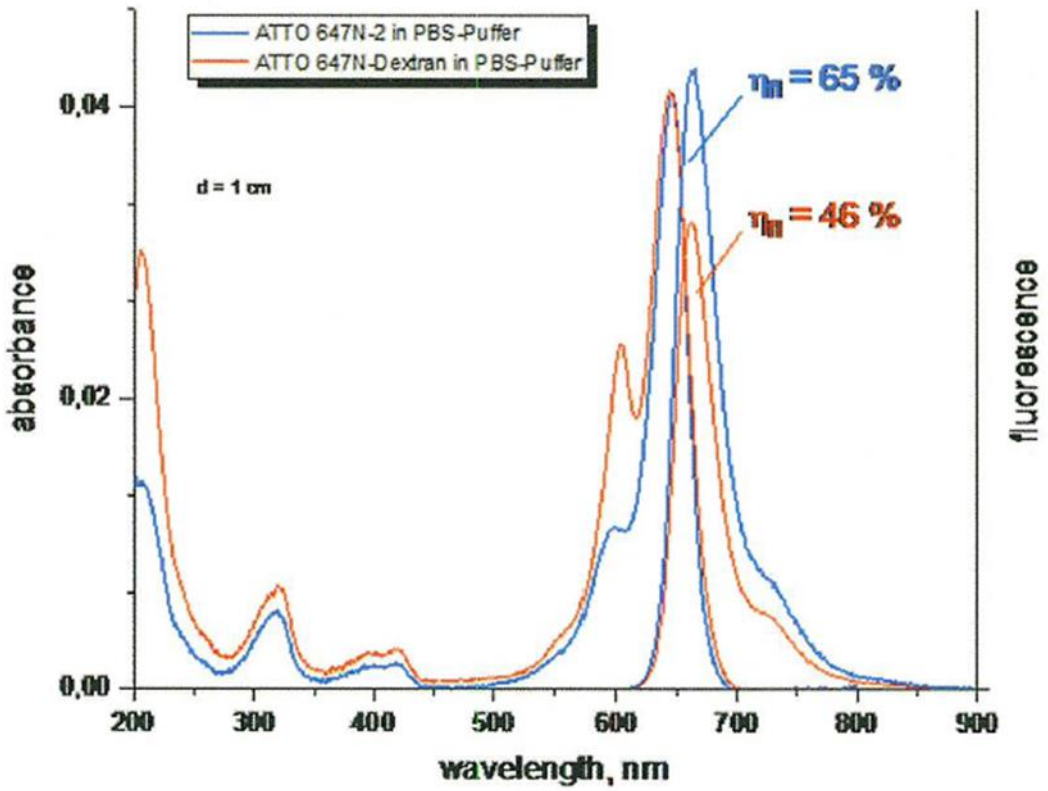
Lysine Dextrans (or Dextran Lysine Fixable) are synthesised from well-characterized dextran fractions derived from Leuconostoc mesenteroides labelled with Lysine. Lysine dextran is then functionalized with Atto647nunder mild conditions to form Atto647n-lysine-dextran. After purification, the products are controlled for Mw, appearance, solubility, DS, pH, free Lysine and free dye. The products are designated by the approximate molecular weights of the dextran fractions used. Thus, for example, the product Atto647n-lysine-dextran 4 has a molecular weight of approx. 4000 Da. The actual molecular weight is determined by GPC. This value is supplied with the Certificate of Analysis. The dextran used is from Leuconostoc mesenteroides B-512F which is essentially a linear α-(1-6)-linked glucose chain with however a low percentage (2-5%) of α-(1-3) branches distributed along the chain. The dextran fractions used are from Mw of 4000 to 500000 and are carefully controlled by GPC, optical rotation, absorbance and other control parameters.

Physical properties

The fluorescent dye Atto647n belongs to a new generation of fluorescent dyes for the red spectral region and exhibits strong absorption, excellent fluorescence quantum yield, high photostability, excellent ozone resistance and very little triplet formation. The dye is suitable for single-molecule detection and high-resolution microscopy. The dye is cationic and carries a net charge of +1 when conjugated with dextran and shows no pH dependence in the range of pH 2-11. Atto647n is a mixture of two isomers with practically identical properties.

Atto647n-lysine-dextrans are blue powders that are readily soluble in water or electrolyte solutions. Atto647n-lysine-dextran are insoluble in most organic solvents, such as ethanol, methanol, acetone,

chloroform, ethyl acetate etc. The degree of substitution, (DS) is between 0.01-0.02 (mol Lysine/mol Glucose) and 0.001-0.01 (mol Atto647n/mol Glucose). Atto647n has an excitation maximum at 646 nm



and an emission maximum at 664 nm (Fig.2).

Fig 2. Absorbance and fluorescence scan of Atto647nLysine Dextran 70 in pH 7.4 buffer. Excitation 646 nm, Emission 664 nm.

Stability

No prospective stability studies on Atto647nLysine Dextrans have been performed yet. However, the structural properties of the dextran and of the carbamide linkage of the Lysine to the dextran chain as well as the amide bond between Lysine and Atto647nwould suggest high stability of the product. It is recommended that the products are stored in air-tight containers. Atto647nLysine Dextrans may be stored at ambient temperatures.

Applications

Dextrans carrying amino groups are valuable to the scientific community as versatile tools for bioconjugation and fixation in living systems[1]. For conjugation, the free amino group of the Lysine structure will be able to covalently bind to activated entities such as NHS-esters or isothiocyanates or be coupled to carboxylic acids using common peptide coupling reagents such as DCC, HOBt and or HATU. Cell and tissue fixation are performed in order to preserve components in a "life-like state" and to make cells permeable to allow antibodies to access cellular structures, for example for microscopy studies or immunostaining. The amino groups on Lysine Dextran reacts with the fixing agent (here:

formaldehyde or glutaraldehyde) to form a covalent crosslinking with biomolecules such as proteins and lipids, immobilizing the whole system and the dextran. This is of particular importance when evaluating biological events qualitatively or quantitatively in molecular imaging. Without fixation, these structures within a living system would fall apart and diffuse rapidly. Atto647nis a red-shifted dye with good tissue penetration properties.

References

1. http://www.atto-tec.com/fileadmin/user_upload/Katalog_Flyer_Support/ATTO_647N.pdf
2. (a) Henley JR, Krueger EW, Oswald BJ, McNiven MA, J Cell Biol (1998) 141:85-99; (b) Fritzsch B, Christensen MA, Nichols DH; J Neurobiol. 1993 Nov;24(11):1481-99; (c) Fritzsch B., J Neurosci Methods (1993) 50:95-103.