# Erratum: AMBER-DYES in AMBER: Implementation of fluorophore and linker parameters into AmberTools

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## Erratum

Previously, we adapted the AMBER-DYES parameter set derived by Graen et al.[1] into "AMBER-DYES in AMBER" to generate a force field applicable within the AMBER package of molecular simulation codes[2] for commonly used fluorescent dyes and linkers attached to a protein[3]. Recently, we became aware that the chemical structure of Alexa Fluor™ 647 (Alexa 647) available in "AMBER-DYES in AMBER" (Figure 1A), which we took over from Graen et al.[1], differs from the chemical structure generally available and now considered correct for Alexa 647[4] (Figure 1B). The chemical structure of Alexa 647 nowadays commercially available from Thermo Fisher Scientific[5] has two differences (Figure 1A, B): I) The length of the carbon chain between the carbonyl group and the indolium moiety of the dye; II) the length of the two carbon chains between the sulfo groups and the indolium moieties. Note that yet other chemical structures of Alexa 647 were depicted in refs. [6] (Figure 1C) and [7] (Figure 1D).

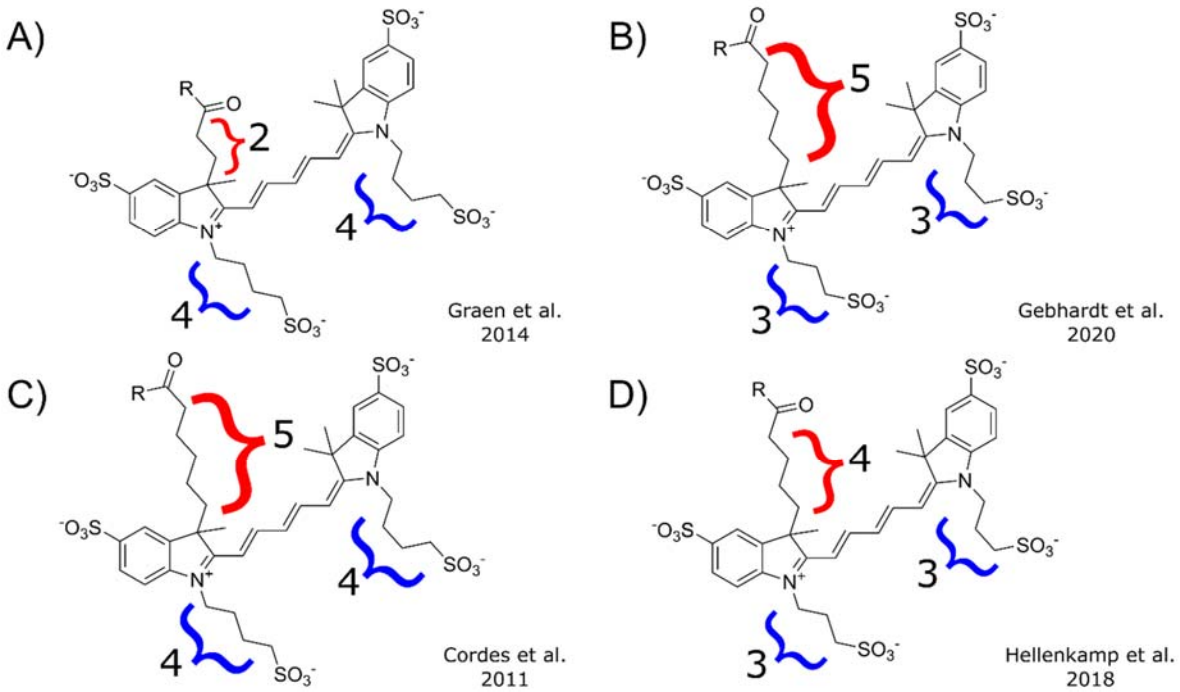


**Figure 1. Structures of Alexa Fluor™ 647 used in publications.** The length of the carbon chain between the carbonyl group and the indolium moiety of the dye is marked by a red bracket; the length of the two carbon chains between the sulfo groups and the indolium moieties is marked by a blue bracket. **A)** Structure used by Graen et al.[1] to parametrize AMBER-Dyes. The structure has a $C_2$-chain (red) and two $C_4$-chains (blue) and agrees with that in ref. [1]. **B)** Correct structure with $C_5$-chain (red) and two $C_3$-chains (blue)

according to refs. [4]. **C)** Structure used in ref. [6] with the correct $C_5$-chain (red), but wrong $C_4$-chains (blue). **D)** Structure used in ref. [7] with wrong $C_4$-chain (red), but correct $C_3$-chains (blue).

For the correct structure (Figure 1B), we derived partial atomic charges using the restrained electrostatic potential (RESP [8]) procedure following the workflow described for the reparameterization of the cysteine linker in ref. [3]. The longer carbon chain between the indolium moiety and the carbonyl group (Figure 1B, red bracket) allows the dye to move more freely and occupy a larger volume around its attachment point.

The updated parameters for the Alexa 647 dye are listed in Texts S1 and S2. A *leaprc.amberdyes* file was created, which, when sourced in LEaP[9], automatically loads the *lib* and *dat* files for the respective dye/linker combinations (see Table 1 in ref. [3]). The updated parameters have been made available in AmberTols20.[2]

ASSOCIATED CONTENT

**Supporting Information**. Supplementary text containing the new parameters for Alexa 647.

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**Notes**

The authors declare no competing financial interest.

ACKNOWLEDGMENT

We are grateful for computational support and infrastructure provided by the "Zentrum für Informations- und Medientechnologie" (ZIM) at the Heinrich Heine University Düsseldorf and the computing time provided by the John von Neumann Institute for Computing (NIC) to H.G. on the supercomputers JURECA and JUWELS at Jülich Supercomputing Centre (JSC, user IDs: HDD201 and schepers1).

ABBREVIATIONS

T4L, T4 Lysozyme; Alexa Fluor 647, Alexa 647

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