Supplemental Information for

Orientation of nitro-group governs the fluorescence lifetime of nitrobenzoxadiazole (NBD)-labeled lipids in lipid bilayers

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Supplementary Information

Contents

Table S1: average lifetimes of C_6NBD-PC and C_{12}NBD-PC in DOPC and POPC LUVs

Average fluorescence lifetime, $\langle\tau\rangle$, of C_6NBD-PC or C_{12}NBD-PC in DOPC or POPC LUVs of different diameter, in H_2O buffer. Error are deviations from at least 3 independent samples.

$\langle\tau\rangle$ (ns)	POPC	DOPC
C_6NBD-PC	7.16 ± 0.18	6.35 ± 0.13
C_{12}NBD-PC	6.21 ± 0.04	5.76 ± 0.05

Topology file for C_6NBD-PC (Gromos 53a6 force field)

```
[ moleculetype ]
; Name   nrexcl
NBD      3

[ atoms ]
;   nr    type   resnr  residu    atom    cgnr         charge      mass
     1    CH3L       1     NBD      C1       1         0.4000   15.0350
     2    CH3L       1     NBD      C2       1         0.4000   15.0350
     3    CH3L       1     NBD      C3       1         0.4000   15.0350
     4      NL       1     NBD      N4       1        -0.5000   14.0067
     5     CH2       1     NBD      C5       1         0.3000   14.0270
     6     CH2       1     NBD      C6       2         0.4000   14.0270
     7      OA       1     NBD      O7       2        -0.8000   15.9994
     8       P       1     NBD      P8       2         1.7000   30.9738
     9     OML       1     NBD      O9       2        -0.8000   15.9994
    10     OML       1     NBD     O10       2        -0.8000   15.9994
    11      OA       1     NBD     O11       2        -0.7000   15.9994
    12     CH2       1     NBD     C12       3         0.4000   14.0270
    13     CH1       1     NBD     C13       3         0.3000   13.0190
    14      OE       1     NBD     O14       3        -0.7000   15.9994
    15       C       1     NBD     C15       3         0.7000   12.0110
    16       O       1     NBD     O16       3        -0.7000   15.9994
    17     CH2       1     NBD     C17       4         0.0000   14.0270
    18     CH2       1     NBD     C18       4         0.0000   14.0270
    19     CH2       1     NBD     C19       5         0.0000   14.0270
    20     CH2       1     NBD     C20       5         0.0000   14.0270
    21     CH2       1     NBD     C21       6         0.25     14.0270
    22      NE       1     NBD     N22       6        -0.34     14.0067
    23       C       1     NBD     C23       6        -0.01     12.0110
    24     CR1       1     NBD     C24       6        -0.12     13.0190
    25     CR1       1     NBD     C25       6         0.19     13.0190
    26       C       1     NBD     C26       6        -0.37     12.0110
    27      NR       1     NBD     N27       6         0.86     14.0067
    28       O       1     NBD     O28       6        -0.51     15.9994
    29       O       1     NBD     O29       6        -0.46     15.9994
    30      NR       1     NBD     N30       6        -0.30     14.0067
    31       C       1     NBD     C31       6         0.41     12.0110
    32       C       1     NBD     C32       6         0.41     12.0110
    33      OE       1     NBD     O33       6         0.02     15.9994
    34      NR       1     NBD     N34       6        -0.32     14.0067
    35       H       1     NBD     H35       6         0.29      1.008
    36     CH2       1     NBD     C36       7         0.5000   14.0270
    37      OE       1     NBD     O37       7        -0.7000   15.9994
    38       C       1     NBD     C38       7         0.8000   12.0110
    39       O       1     NBD     O39       7        -0.6000   15.9994
    40     CH2       1     NBD     C40       8         0.0000   14.0270
    41     CH2       1     NBD     C41       8         0.0000   14.0270
    42     CH2       1     NBD     C42       9         0.0000   14.0270
    43     CH2       1     NBD     C43       9         0.0000   14.0270
    44     CH2       1     NBD     C44      10         0.0000   14.0270
    45     CH2       1     NBD     C45      10         0.0000   14.0270
    46     CH2       1     NBD     C46      11         0.0000   14.0270
    47     CH2       1     NBD     C47      11         0.0000   14.0270
```

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    49     CH2       1     NBD     C49      12          0.0000   14.0270
    50     CH2       1     NBD     C50      13          0.0000   14.0270
    51     CH2       1     NBD     C51      13          0.0000   14.0270
    52     CH2       1     NBD     C52      14          0.0000   14.0270
    53     CH2       1     NBD     C53      14          0.0000   14.0270
    54     CH3       1     NBD     C54      15          0.0000   15.0350

[ bonds ]
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       8      11       2  gb_28
      11      12       2  gb_18
      12      13       2  gb_27
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      23      24       2  0.13940E+00 1.0800e+07
      23      32       2  0.14440E+00 1.0800e+07
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      25      26       2  0.13800E+00 1.0800e+07
      26      27       2  0.14450E+00 8.5400e+06
      26      31       2  0.14310E+00 1.0800e+07
      27      28       2  0.12370E+00 1.6600e+07
      27      29       2  0.12320E+00 1.6600e+07
      30      31       2  0.13240E+00 1.1800e+07
      30      33       2  0.13780E+00 1.1000e+07
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      32      34       2  0.13160E+00 1.1800e+07
      33      34       2  0.13600E+00 1.1000e+07
      21      22       2  0.14570E+00 8.7100e+06
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[ pairs ]
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    7    12     1
    8    13     1
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   10    12     1
   11    14     1
   11    36     1
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   25    30     1
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   37    41     1
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   40    43     1
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   43    46     1
   44    47     1
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   47    50     1
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   50    53     1
   51    54     1

[ angles ]
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        2       4       5       2  ga_13
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        4       5       6       2  ga_15
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        7       8       9       2  ga_14
        7       8      10       2  ga_14
        7       8      11       2  ga_5
        8      11      12       2  ga_26
        9       8      10       2  ga_29
        9       8      11       2  ga_14
       10       8      11       2  ga_14
       11      12      13       2  ga_15
       12      13      14       2  ga_13
       12      13      36       2  ga_13
       13      14      15       2  ga_22
       13      36      37       2  ga_13
       14      13      36       2  ga_13
       14      15      16       2  ga_33
       14      15      17       2  ga_16
       15      17      18       2  ga_15
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      22      23      32       2 119.00      750.00
      23      22      35       2 117.20      390.00
      23      24      25       2 121.40      560.00
      23      32      31       2 123.30      560.00
      23      32      34       2 127.30      560.00
      24      23      32       2 115.50      560.00
      24      25      26       2 123.90      560.00
      25      26      27       2 120.10      560.00
      25      26      31       2 117.70      560.00
      26      27      28       2 118.00      750.00
      26      27      29       2 117.30      750.00
      26      31      30       2 133.60      560.00
      26      31      32       2 118.30      560.00
      27      26      31       2 122.20      560.00
      28      27      29       2 124.80      750.00
      30      31      32       2 108.10      465.00
      30      33      34       2 113.10      450.00
      31      30      33       2 104.70      465.00
      31      32      34       2 109.40      465.00
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      21      22      35       2 118.60      460.00
      21      22      23       2 124.00      730.00
      20      21      22       2 111.10      530.00
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      38      40      41       2  ga_15
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      42      43      44       2  ga_15
      43      44      45       2  ga_15
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      51      52      53       2  ga_15
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[ dihedrals ]
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    4     5     6     7     1   gd_36
    5     6     7     8     1   gd_29
    6     7     8    11     1   gd_20
    6     7     8    11     1   gd_27
    7     8    11    12     1   gd_20
    7     8    11    12     1   gd_27
    8    11    12    13     1   gd_29
   11    12    13    36     1   gd_34
```

```
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   12    13    36    37     1   gd_34
   13    14    15    17     1   gd_13
   13    36    37    38     1   gd_29
   14    15    17    18     1   gd_40
   15    17    18    19     1   gd_34
   17    18    19    20     1   gd_34
   18    19    20    21     1   gd_34
   19    20    21    22     1    gd_34
   20    21    22    35     1    gd_41
   21    22    23    24     1    0.0    33.50  2
   31    26    27    29     1    0.0    33.50  2
   36    37    38    40     1   gd_13
   37    38    40    41     1   gd_40
   38    40    41    42     1   gd_34
   40    41    42    43     1   gd_34
   41    42    43    44     1   gd_34
   42    43    44    45     1   gd_34
   43    44    45    46     1   gd_34
   44    45    46    47     1   gd_34
   45    46    47    48     1   gd_34
   46    47    48    49     1   gd_34
   47    48    49    50     1   gd_34
   48    49    50    51     1   gd_34
   49    50    51    52     1   gd_34
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   51    52    53    54     1   gd_34

[ dihedrals ]
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      23      22      32      24       2 0.00000E+00 0.16740E+03
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      26      31      27      25       2 0.00000E+00 0.16740E+03
      27      26      29      28       2 0.00000E+00 0.16740E+03
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      30      31      32      34       2 0.00000E+00 0.16740E+03
      31      32      34      33       2 0.00000E+00 0.16740E+03
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      32      31      26      25       2 0.00000E+00 0.16740E+03
      31      26      25      24       2 0.00000E+00 0.16740E+03
      26      25      24      23       2 0.00000E+00 0.16740E+03
      25      24      23      32       2 0.00000E+00 0.16740E+03
      24      23      32      31       2 0.00000E+00 0.16740E+03
      29      27      26      31       2 0.00000E+00 0.16740E+03
      25      26      27      28       2 0.00000E+00 0.16740E+03
```

Topology file for C_{12}NBD-PC (Gromos 53a6 force field)

```
[ moleculetype ]
; Name   nrexcl
NBD      3

[ atoms ]
;   nr    type   resnr  residu    atom    cgnr         charge      mass
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     2    CH3L       1     NBD      C2       1         0.4000   15.0350
     3    CH3L       1     NBD      C3       1         0.4000   15.0350
     4      NL       1     NBD      N4       1        -0.5000   14.0067
     5     CH2       1     NBD      C5       1         0.3000   14.0270
     6     CH2       1     NBD      C6       2         0.4000   14.0270
     7      OA       1     NBD      O7       2        -0.8000   15.9994
     8       P       1     NBD      P8       2         1.7000   30.9738
     9     OML       1     NBD      O9       2        -0.8000   15.9994
    10     OML       1     NBD     O10       2        -0.8000   15.9994
    11      OA       1     NBD     O11       2        -0.7000   15.9994
    12     CH2       1     NBD     C12       3         0.4000   14.0270
    13     CH1       1     NBD     C13       3         0.3000   13.0190
    14      OE       1     NBD     O14       3        -0.7000   15.9994
    15       C       1     NBD     C15       3         0.7000   12.0110
    16       O       1     NBD     O16       3        -0.7000   15.9994
    17     CH2       1     NBD     C17       4         0.0000   14.0270
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    20     CH2       1     NBD     C20       5         0.0000   14.0270
    21     CH2       1     NBD     C21       6         0.0000   14.0270
    22     CH2       1     NBD     C22       6         0.0000   14.0270
    23     CH2       1     NBD     C23       7         0.0000   14.0270
    24     CH2       1     NBD     C24       7         0.0000   14.0270
    25     CH2       1     NBD     C25       8         0.0000   14.0270
    26     CH2       1     NBD     C26       8         0.0000   14.0270
    27     CH2       1     NBD     C27       9         0.25     14.0270
    28      NE       1     NBD     N28       9        -0.34     14.0067
    29       C       1     NBD     C29       9        -0.01     12.0110
    30     CR1       1     NBD     C30       9        -0.12     13.0190
    31     CR1       1     NBD     C31       9         0.19     13.0190
    32       C       1     NBD     C32       9        -0.37     12.0110
    33      NR       1     NBD     N33       9         0.86     14.0067
    34       O       1     NBD     O34       9        -0.51     15.9994
    35       O       1     NBD     O35       9        -0.46     15.9994
    36      NR       1     NBD     N36       9        -0.30     14.0067
    37       C       1     NBD     C37       9         0.41     12.0110
    38       C       1     NBD     C38       9         0.41     12.0110
    39      OE       1     NBD     O39       9         0.02     15.9994
    40      NR       1     NBD     N40       9        -0.32     14.0067
    41       H       1     NBD     H41       9         0.29      1.008
    42     CH2       1     NBD     C42      10         0.5000   14.0270
    43      OE       1     NBD     O43      10        -0.7000   15.9994
    44       C       1     NBD     C44      10         0.8000   12.0110
    45       O       1     NBD     O45      10        -0.6000   15.9994
    46     CH2       1     NBD     C46      11         0.0000   14.0270
    47     CH2       1     NBD     C47      11         0.0000   14.0270
```

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    48     CH2       1     NBD     C48      12          0.0000   14.0270
    49     CH2       1     NBD     C49      12          0.0000   14.0270
    50     CH2       1     NBD     C50      13          0.0000   14.0270
    51     CH2       1     NBD     C51      13          0.0000   14.0270
    52     CH2       1     NBD     C52      14          0.0000   14.0270
    53     CH2       1     NBD     C53      14          0.0000   14.0270
    54     CH2       1     NBD     C54      15          0.0000   14.0270
    55     CH2       1     NBD     C55      15          0.0000   14.0270
    56     CH2       1     NBD     C56      16          0.0000   14.0270
    57     CH2       1     NBD     C57      16          0.0000   14.0270
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    59     CH2       1     NBD     C59      17          0.0000   14.0270
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[ bonds ]
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       3       4       2  gb_21
       4       5       2  gb_21
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       7       8       2  gb_28
       8       9       2  gb_24
       8      10       2  gb_24
       8      11       2  gb_28
      11      12       2  gb_18
      12      13       2  gb_27
      13      14       2  gb_18
      13      42       2  gb_27
      14      15       2  gb_10
      15      16       2  gb_5
      15      17       2  gb_23
      17      18       2  gb_27
      18      19       2  gb_27
      19      20       2  gb_27
      20      21       2  gb_27
      21      22       2  gb_27
      22      23       2  gb_27
      23      24       2  gb_27
      24      25       2  gb_27
      25      26       2  gb_27
      26      27       2  gb_27
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      29      30       2  0.13940E+00 1.0800e+07
      29      38       2  0.14440E+00 1.0800e+07
      30      31       2  0.14140E+00 1.0800e+07
      31      32       2  0.13800E+00 1.0800e+07
      32      33       2  0.14450E+00 8.5400e+06
      32      37       2  0.14310E+00 1.0800e+07
      33      34       2  0.12370E+00 1.6600e+07
      33      35       2  0.12320E+00 1.6600e+07
      36      37       2  0.13240E+00 1.1800e+07
      36      39       2  0.13780E+00 1.1000e+07
      37      38       2  0.14370E+00 1.0800e+07
```

```
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      39      40       2  0.13600E+00 1.1000e+07
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      43      44       2  gb_10
      44      45       2  gb_5
      44      46       2  gb_23
      46      47       2  gb_27
      47      48       2  gb_27
      48      49       2  gb_27
      49      50       2  gb_27
      50      51       2  gb_27
      51      52       2  gb_27
      52      53       2  gb_27
      53      54       2  gb_27
      54      55       2  gb_27
      55      56       2  gb_27
      56      57       2  gb_27
      57      58       2  gb_27
      58      59       2  gb_27
      59      60       2  gb_27

[ pairs ]
;  ai    aj funct
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    2     6     1
    3     6     1
    4     7     1
    5     8     1
    6     9     1
    6    10     1
    6    11     1
    7    12     1
    8    13     1
    9    12     1
   10    12     1
   11    14     1
   11    42     1
   12    15     1
   12    43     1
   13    16     1
   13    17     1
   13    44     1
   14    18     1
   14    43     1
   15    19     1
   15    42     1
   16    18     1
   17    20     1
   18    21     1
   19    22     1
   20    23     1
   21    24     1
   22    25     1
   23    26     1
   24    27     1
```

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   28    40     1
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   29    32     1
   29    36     1
   29    39     1
   30    33     1
   30    37     1
   30    40     1
   30    41     1
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   31    35     1
   31    36     1
   31    38     1
   32    39     1
   32    40     1
   33    36     1
   33    38     1
   34    37     1
   35    37     1
   38    41     1
   25    28     1
   27    30     1
   27    38     1
   26    29     1
   26    41     1
   42    45     1
   42    46     1
   43    47     1
   44    48     1
   45    47     1
   46    49     1
   47    50     1
   48    51     1
   49    52     1
   50    53     1
   51    54     1
   52    55     1
   53    56     1
   54    57     1
   55    58     1
   56    59     1
   57    60     1

[ angles ]
;  ai    aj    ak funct
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        1       4       3       2  ga_13
        1       4       5       2  ga_13
        2       4       3       2  ga_13
        2       4       5       2  ga_13
        3       4       5       2  ga_13
        4       5       6       2  ga_15
        5       6       7       2  ga_15
        6       7       8       2  ga_26
        7       8       9       2  ga_14
```

7	8	10	2	ga_14	
7	8	11	2	ga_5	
8	11	12	2	ga_26	
9	8	10	2	ga_29	
9	8	11	2	ga_14	
10	8	11	2	ga_14	
11	12	13	2	ga_15	
12	13	14	2	ga_13	
12	13	42	2	ga_13	
13	14	15	2	ga_22	
13	42	43	2	ga_13	
14	13	42	2	ga_13	
14	15	16	2	ga_33	
14	15	17	2	ga_16	
15	17	18	2	ga_15	
16	15	17	2	ga_30	
17	18	19	2	ga_15	
18	19	20	2	ga_15	
19	20	21	2	ga_15	
20	21	22	2	ga_15	
21	22	23	2	ga_15	
22	23	24	2	ga_15	
23	24	25	2	ga_15	
24	25	26	2	ga_15	
25	26	27	2	ga_15	
28	29	30	2	125.60	750.00
28	29	38	2	119.00	750.00
29	28	41	2	117.20	390.00
29	30	31	2	121.40	560.00
29	38	37	2	123.30	560.00
29	38	40	2	127.30	560.00
30	29	38	2	115.50	560.00
30	31	32	2	123.90	560.00
31	32	33	2	120.10	560.00
31	32	37	2	117.70	560.00
32	33	34	2	118.00	750.00
32	33	35	2	117.30	750.00
32	37	36	2	133.60	560.00
32	37	38	2	118.30	560.00
33	32	37	2	122.20	560.00
34	33	35	2	124.80	750.00
36	37	38	2	108.10	465.00
36	39	40	2	113.10	450.00
37	36	39	2	104.70	465.00
37	38	40	2	109.40	465.00
38	40	39	2	104.70	465.00
27	28	41	2	118.60	460.00
27	28	29	2	124.00	730.00
26	27	28	2	111.10	530.00
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43	44	45	2	ga_33	
43	44	46	2	ga_16	
44	46	47	2	ga_15	
45	44	46	2	ga_30	
46	47	48	2	ga_15	
47	48	49	2	ga_15	

```
      48      49      50       2  ga_15
      49      50      51       2  ga_15
      50      51      52       2  ga_15
      51      52      53       2  ga_15
      52      53      54       2  ga_15
      53      54      55       2  ga_15
      54      55      56       2  ga_15
      55      56      57       2  ga_15
      56      57      58       2  ga_15
      57      58      59       2  ga_15
      58      59      60       2  ga_15

[ dihedrals ]
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    4     5     6     7     1   gd_36
    5     6     7     8     1   gd_29
    6     7     8    11     1   gd_20
    6     7     8    11     1   gd_27
    7     8    11    12     1   gd_20
    7     8    11    12     1   gd_27
    8    11    12    13     1   gd_29
   11    12    13    42     1   gd_34
   12    13    14    15     1   gd_29
   12    13    42    43     1   gd_34
   13    14    15    17     1   gd_13
   13    42    43    44     1   gd_29
   14    15    17    18     1   gd_40
   15    17    18    19     1   gd_34
   17    18    19    20     1   gd_34
   18    19    20    21     1   gd_34
   19    20    21    22     1   gd_34
   20    21    22    23     1   gd_34
   21    22    23    24     1   gd_34
   22    23    24    25     1   gd_34
   23    24    25    26     1   gd_34
   24    25    26    27     1   gd_34
   25    26    27    28     1    gd_34
   26    27    28    41     1    gd_41
   27    28    29    30     1    0.0    33.50  2
   37    32    33    35     1    0.0    33.50  2
   42    43    44    46     1   gd_13
   43    44    46    47     1   gd_40
   44    46    47    48     1   gd_34
   46    47    48    49     1   gd_34
   47    48    49    50     1   gd_34
   48    49    50    51     1   gd_34
   49    50    51    52     1   gd_34
   50    51    52    53     1   gd_34
   51    52    53    54     1   gd_34
   52    53    54    55     1   gd_34
   53    54    55    56     1   gd_34
   54    55    56    57     1   gd_34
   55    56    57    58     1   gd_34
   56    57    58    59     1   gd_34
```

```
   57    58    59    60     1   gd_34

[ dihedrals ]
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       13       14       42       12        2   gi_2
       15       14       16       17        2   gi_1
       44       43       45       46        2   gi_1
       28       27       41       29        2 0.00000E+00 0.16740E+03
       29       28       38       30        2 0.00000E+00 0.16740E+03
       38       29       37       40        2 0.00000E+00 0.16740E+03
       37       38       32       36        2 0.00000E+00 0.16740E+03
       32       37       33       31        2 0.00000E+00 0.16740E+03
       33       32       35       34        2 0.00000E+00 0.16740E+03
       38       40       39       36        2 0.00000E+00 0.16740E+03
       40       39       36       37        2 0.00000E+00 0.16740E+03
       39       36       37       38        2 0.00000E+00 0.16740E+03
       36       37       38       40        2 0.00000E+00 0.16740E+03
       37       38       40       39        2 0.00000E+00 0.16740E+03
       29       38       37       32        2 0.00000E+00 0.16740E+03
       38       37       32       31        2 0.00000E+00 0.16740E+03
       37       32       31       30        2 0.00000E+00 0.16740E+03
       32       31       30       29        2 0.00000E+00 0.16740E+03
       31       30       29       38        2 0.00000E+00 0.16740E+03
       30       29       38       37        2 0.00000E+00 0.16740E+03
       35       33       32       37        2 0.00000E+00 0.16740E+03
       31       32       33       34        2 0.00000E+00 0.16740E+03
```

Topology file for C_4NBD-PC (Martini 2.0 force field)

```
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; molname      nrexcl
  NC04       1

[atoms]
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   2  Qa     1    NC04  PO4    2    -1.0
   3  Na     1    NC04  GL1    3    0
   4  Na     1    NC04  GL2    4    0
   5  C2     1    NC04  C1A    5    0
   6  SNda   1    NC04  NHC    4    0
   7  C1     1    NC04  C1B    7    0
   8  C1     1    NC04  C2B    8    0
   9  C1     1    NC04  C3B    9    0
  10  C1     1    NC04  C4B   10    0
  11  SP3    1    NC04  NO2   11    0
  12  SC4    1    NC04  CC1   12    0
  13  SNa    1    NC04  NON   13    0
  14  SC4    1    NC04  CC2   14    0


[bonds]
;  i   j    funct      length     force.c.
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   2   3    1     0.47  1250
   3   4    1     0.37  1250
   3   5    1     0.47  1250
   5   6    1     0.34  1250
   4   7    1     0.47  1250
   7   8    1     0.47  1250
   8   9    1     0.47  1250
   9  10    1     0.47  1250

[constraints]
;  i     j   funct   length
  11  12    1     0.317
  11  14    1     0.311
  12  13    1     0.153
   6  12    1     0.251
   6  14    1     0.247

[angles]
;  i   j   k       funct      angle      force.c.
   2   3   4       2     120.0      25.0
   2   3   5       2     180.0      25.0
   3   5   6       2     180.0      25.0
   4   7   8       2     180.0      25.0
   7   8   9       2     180.0      25.0
   8   9  10       2     180.0      25.0
  11  12  13       2     116.0    1800.0
  11  14   6       2     130.0    2400.0
  12   6  14       2      58.0    6000.0
```

```
  13 12  6       2     117.5    1900.0
  14  6  5       2     135.0      25.0

[dihedrals]
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   12    6    14   11      2    0.0    25.0
   6     14   11   12      2    0.0    25.0
   14    11   12   6       2    0.0    25.0
   12    11   13   6       2    0.0    200.0

[exclusions]
11  12  13   6  14
12  13   6  14
13   6  14
 6  14
12  14
```

Topology file for C_{12}NBD-PC (Martini 2.0 force field)

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   3  Na     1    NC12  GL1    3    0
   4  Na     1    NC12  GL2    4    0
   5  C1     1    NC12  C1A    5    0
   6  C1     1    NC12  C2A    6    0
   7  C2     1    NC12  C3A    7    0
   8  SNda   1    NC12  NHC    8    0
   9  C1     1    NC12  C1B    9    0
  10  C1     1    NC12  C2B   10    0
  11  C1     1    NC12  C3B   11    0
  12  C1     1    NC12  C4B   12    0
  13  SP3    1    NC12  NO2   13    0
  14  SC4    1    NC12  CC1   14    0
  15  SNa    1    NC12  NON   15    0
  16  SC4    1    NC12  CC2   16    0


[bonds]
;  i   j    funct      length     force.c.
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   2   3    1     0.47  1250
   3   4    1     0.37  1250
   3   5    1     0.47  1250
   5   6    1     0.47  1250
   6   7    1     0.47  1250
   7   8    1     0.34  1250
   4   9    1     0.47  1250
   9  10    1     0.47  1250
  10  11    1     0.47  1250
  11  12    1     0.47  1250

[constraints]
;  i     j   funct   length
  13  14    1     0.317
  13  16    1     0.311
  14  15    1     0.153
   8  14    1     0.251
   8  16    1     0.247

[angles]
;  i   j   k       funct      angle      force.c.
   2   3   4       2     120.0      25.0
   2   3   5       2     180.0      25.0
   3   5   6       2     180.0      25.0
   5   6   7       2     180.0      25.0
   6   7   8       2     180.0      25.0
```

```
   4   9 10       2     180.0      25.0
   9  10 11       2     180.0      25.0
  10  11 12       2     180.0      25.0
  13  14 15       2     116.0      1800.0
  13  16 8        2     130.0    2400.0
  14  8 16        2      58.0    6000.0
  15  14 8        2     117.5    1900.0
  16  8 7         2     135.0      25.0

[dihedrals]
;  i     j     k     l   funct   angle  force.c.
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   14    8     16    13      2     0.0    25.0
   8     16    13    14      2     0.0    25.0
   16    13    14    8       2     0.0    25.0
   14    13    15    8       2     0.0    200.0

[exclusions]
13  14  15   8  16
14  15   8  16
15   8  16
 8  16
14  16
```

Description of the Martini parameterization of the NBD fluorophore

The number and distribution of the Martini beads, i.e. mapping, was chosen in order to preserve the geometrical structure of the NBD, which could be done with a five bead configuration, as shown in Fig. S1. Then, the attributed bonded and non-bonded (i.e. bead types) parameters, defined based on the mapping process, were tested and validated comparing the coarse grained (CG) model to atomistic data for the probes composed by the NBD group attached to an alkyl chains of different sizes, NBD-C_4 and NBD-C_8.[1, 2] The bonded parameters were validated comparing 10 ns simulations of the molecule NBD-C_8 in water at both resolution levels. As shown in Fig. S2 to S4, the bond, angle and dihedral distribution of the CG model matched very well the atomistic data. Then, the non-bonded parameters were validated against our previous reported potential of mean force (PMF) profiles for the NBD-C_n homologous series,[2] in this case for both NBD-C_4 and NBD-C_8. The sampling at each umbrella window was done during 200 ns, discarding the first 50 ns as equilibration time. A time step of 20 fs was used in all simulations. As shown in Fig. S5, the PMFs obtained with the CG parameterizations are similar to those obtained at atomistic level, validating the type of Martini beads used.

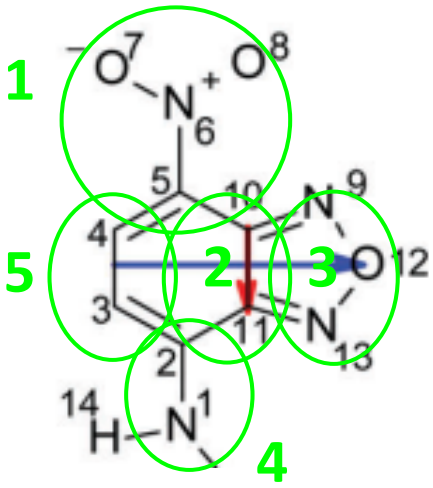

Fig. S1 – Coarse grained mapping of the NBD fluorophore for the Martini parameterization regarding its atomistic structure. The groups of atoms (5, 6, 7, 8), (10, 11), (9, 12, 13), (1,2) and (3, 4) were defined as SP3, SC4, SNa, SNda, and SC4 Martini beads, respectively. Bead numbers are indicated in green.

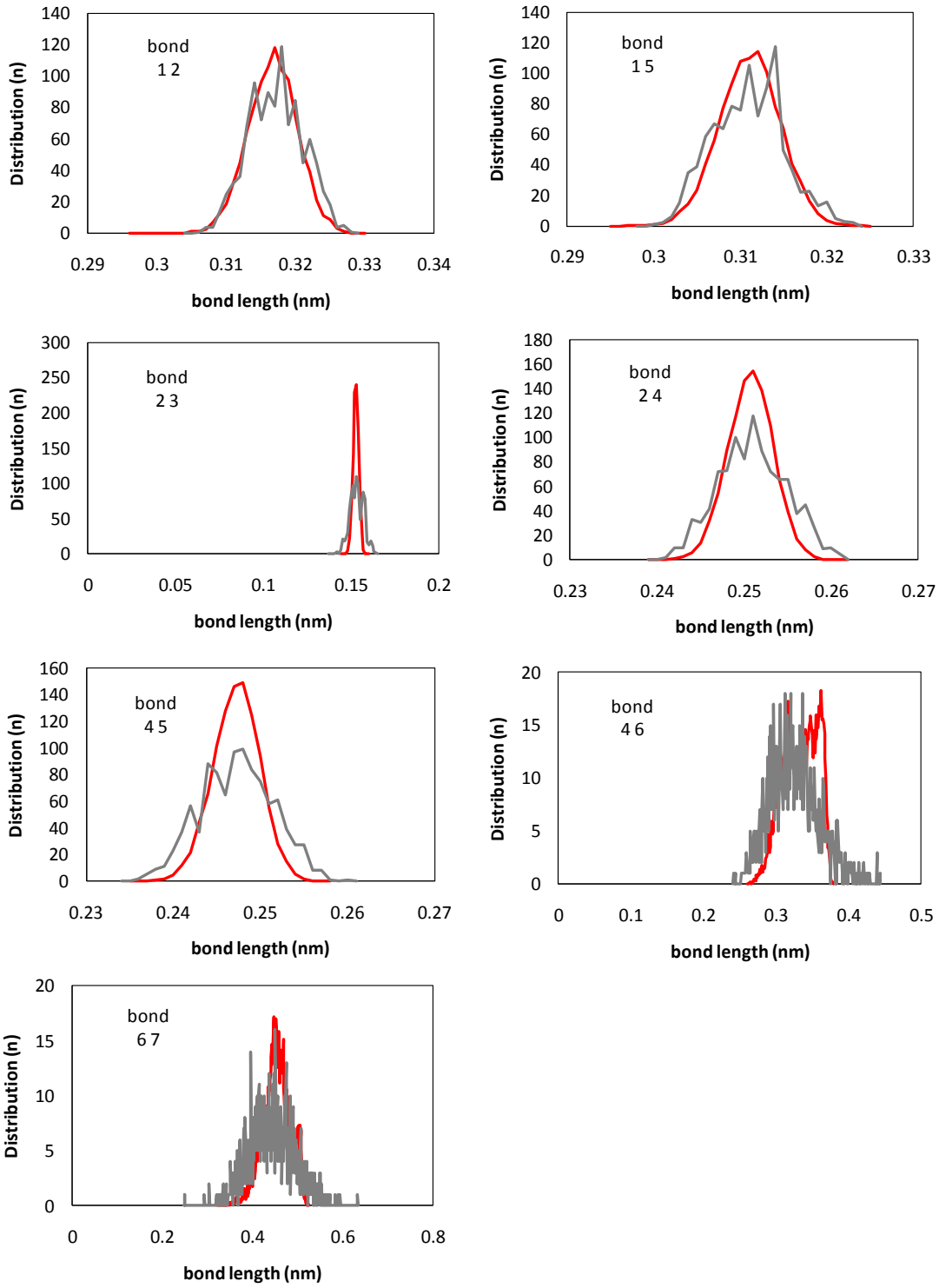

Fig. S2 – Comparison of bond length distributions for the martini (gray) and mapped G53A6FF (red) parameterization of NBD-C_8 as used in reference [1]. For bond identification, please refer to bead number in Figure S1. Bonds (1-2), (1-5), (2-3), (2-4) and (4-5) were set to constraints and bonds (4-6) and (6-7) were set as regular bonds.

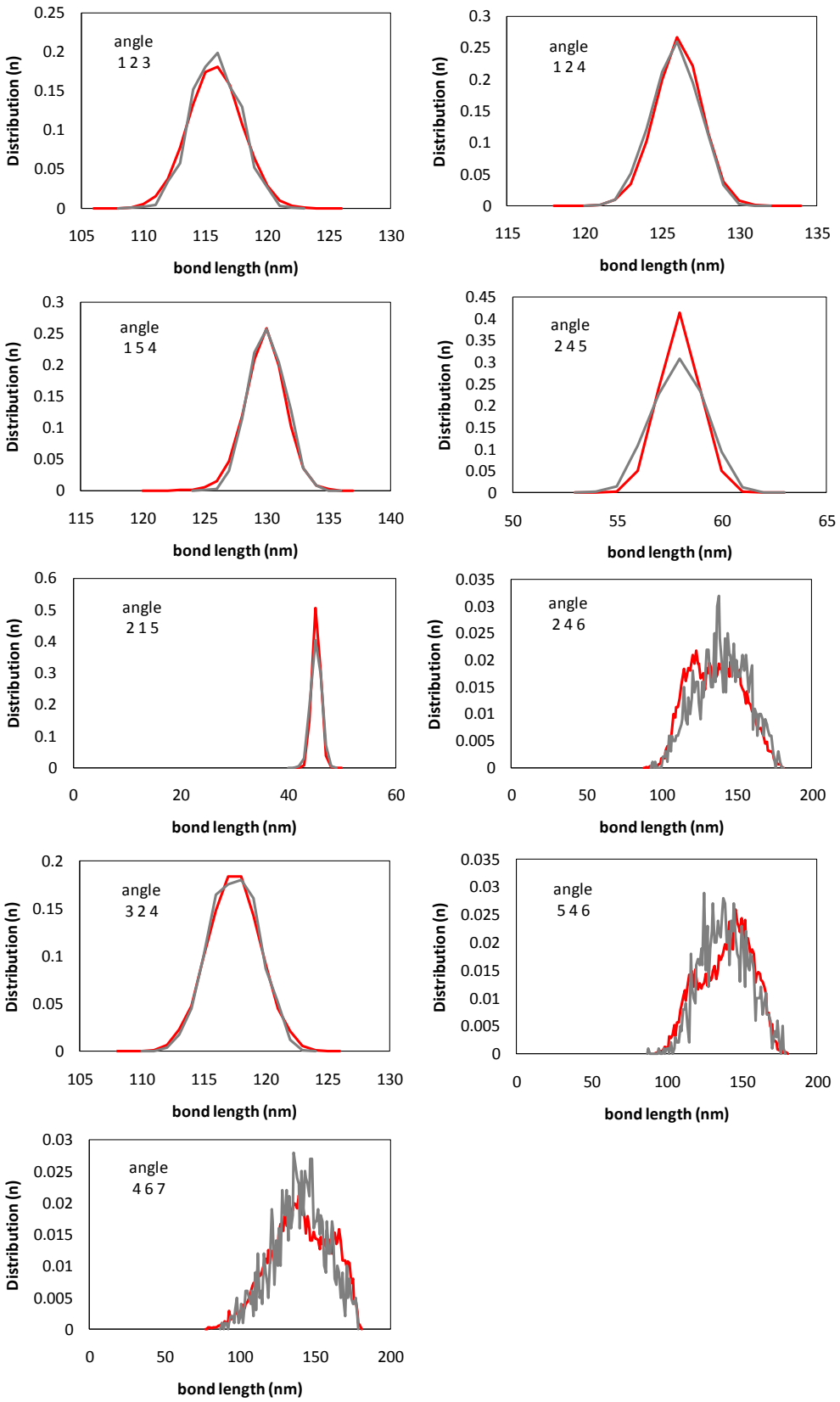

Fig. S3 – Comparison of angle distributions for the martini (gray) and mapped G53A6FF (red) parameterization of NBD-C_8 as used in reference [1]. For angle identification, please refer to bead number in Figure S1. Note that angles (1-2-4), (2-1-5) and (2-4-6) are actually not defined in the parametrization file.

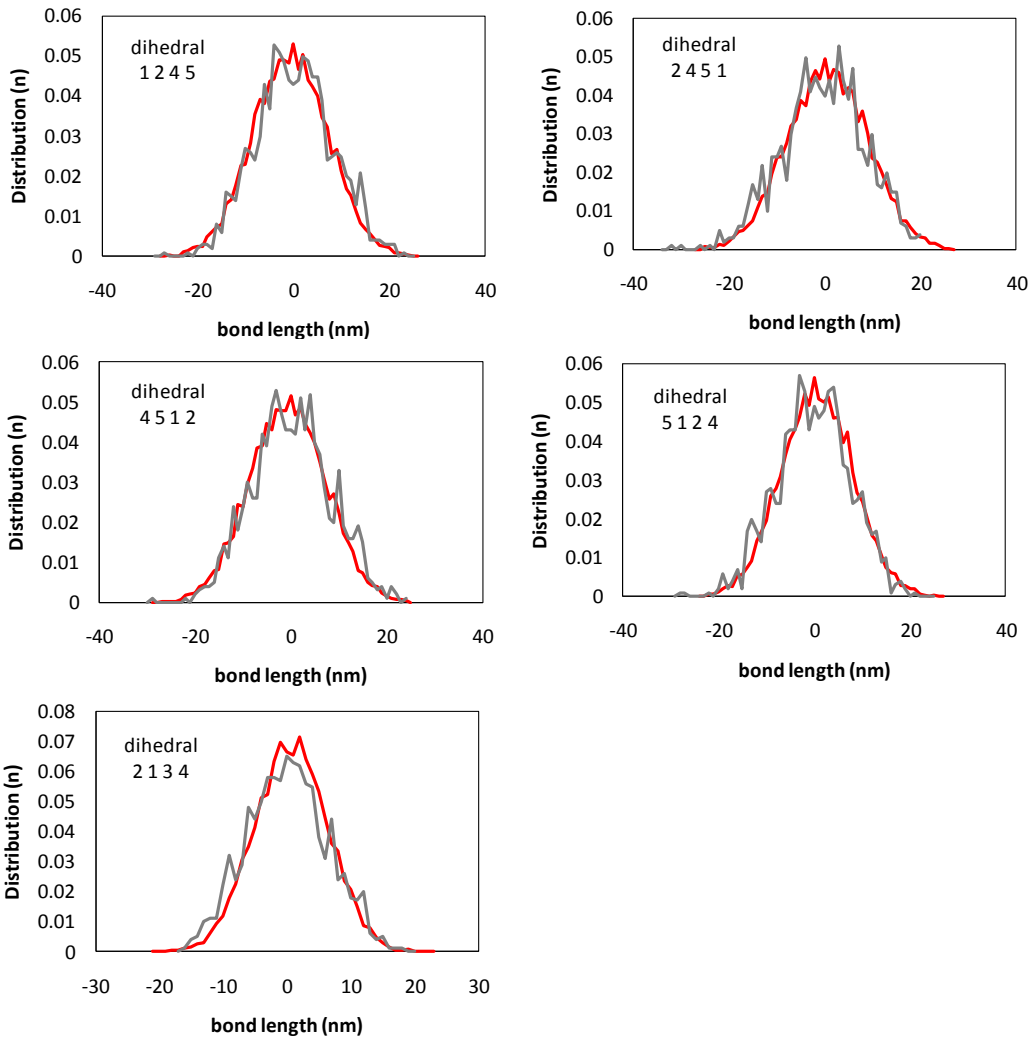

Fig. S4 – Comparison of bond dihedral distributions for the martini (gray) and mapped G53A6FF (red) parameterization of NBD-C_8 as used in reference [1]. For dihedral identification, please refer to bead number in Figure S1.

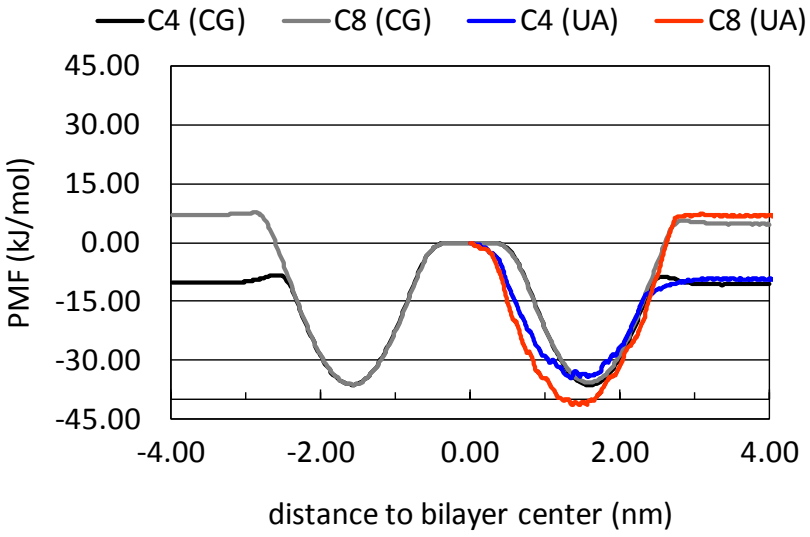

Fig. S5 – Potential of Mean Force (PMF) profiles for the interaction of NBD-C_4 and NBD-C_8 with a POPC bilayer at atomistic [2] and coarse grained (this work) level.

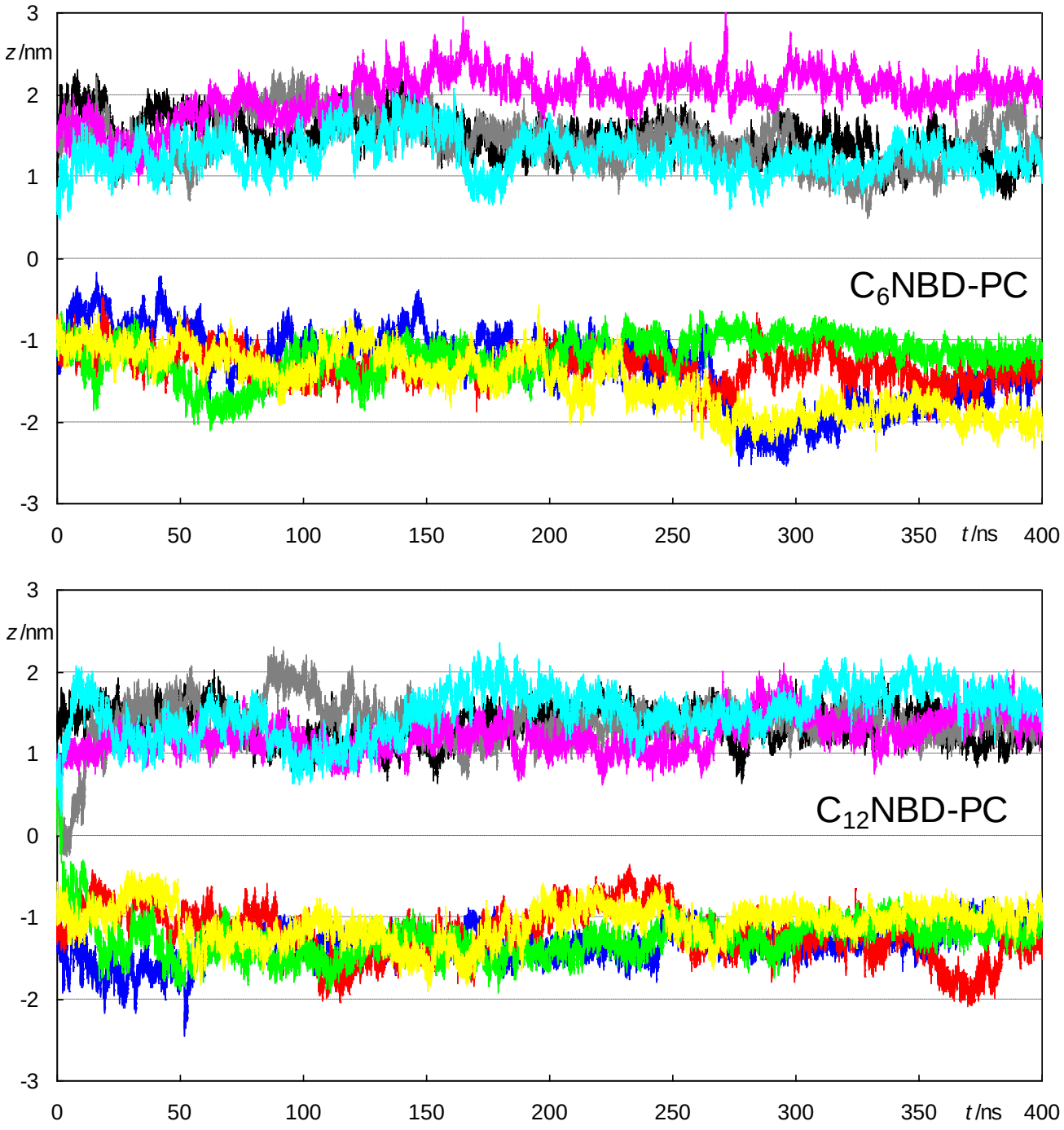

Fig. S6 - Time evolution of the center of mass of the fluorophores of the eight NBD-PC molecules considered in the atomistic simulations, each of them depicted in a distinct color. Top: C_6NBD-PC; bottom: C_{12}NBD-PC.

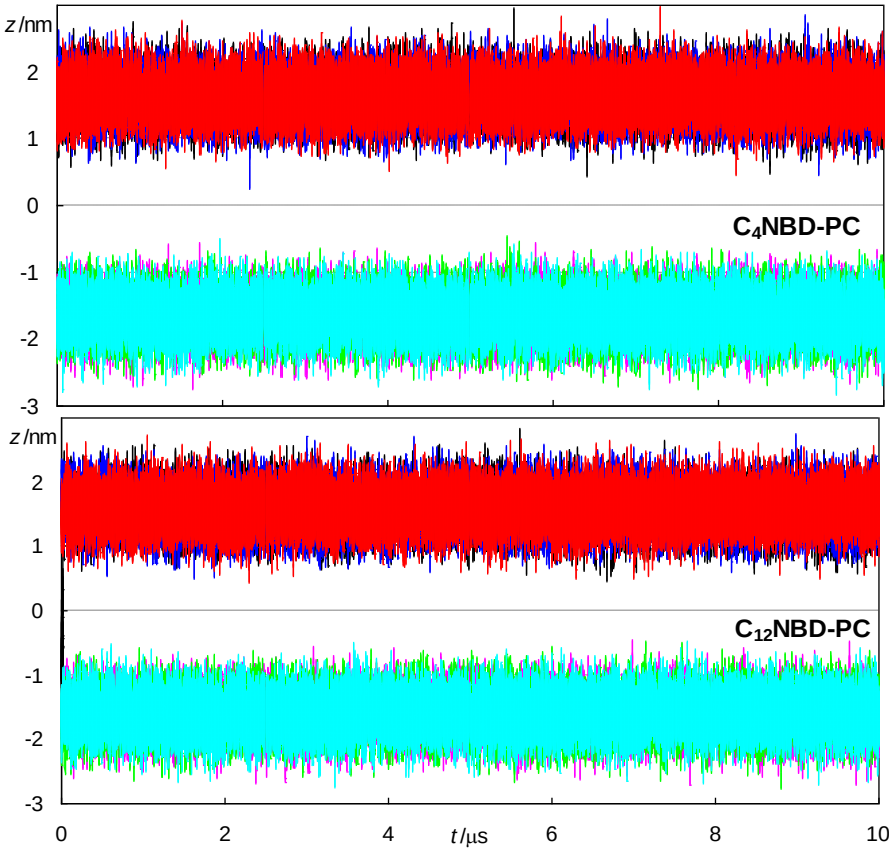

Fig. S7 - Time evolution of the transverse location *z* of the center of mass of the NBD fluorophore of each individual probe (black, blue and red for the top leaflet molecules; pink, green and cyan for the bottom leaflet molecules) in the CG simulations of C_4NBD-PC (top) and C_{12}NBD-PC (bottom).

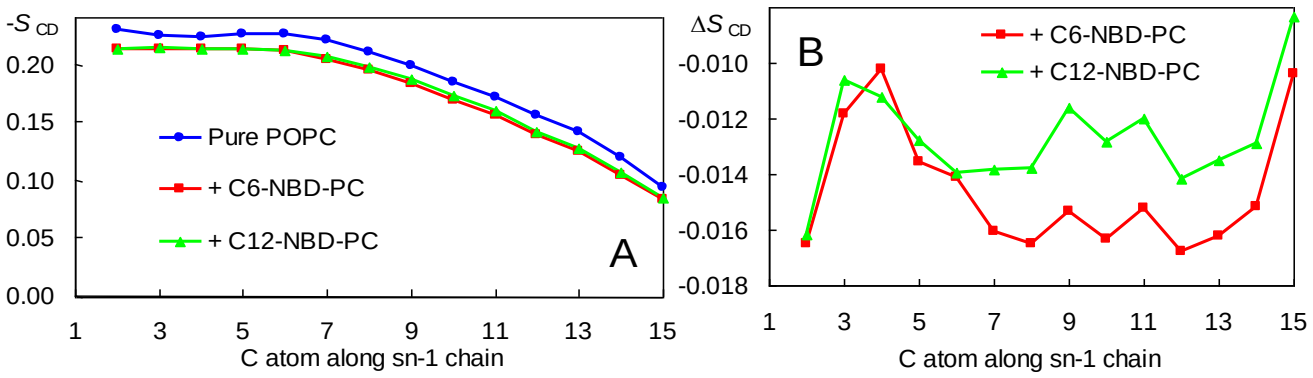

Fig. S8 - (A) Deuterium order parameters (S_{CD}) for the POPC *sn*-1 atoms in the absence and in the presence of C_6- or C_{12}NBD-PC, calculated from the atomistic simulations. (B) Difference order parameter plots (ΔS_{CD}), obtained by subtraction of the pure POPC order parameter profile from those calculated in the presence of C_6- or C_{12}NBD-PC.

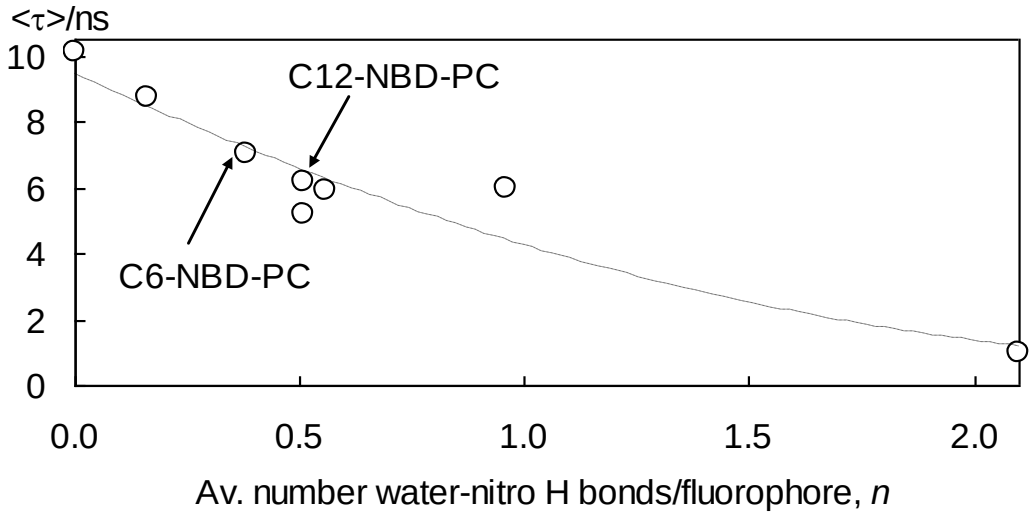

Fig. S9 - Intensity-averaged lifetimes <τ> obtained for different NBD probes in fluid PC bilayers,[3-7] represented as a function of their average number of water-NBD NO_2 hydrogen bonds determined by MD simulation.[3, 8-10] The point (0, 10.1 ns) corresponds to polar aprotic solvents such as acetone, tetrahydrofuran or dioxane [11]. For water, <τ> = 1.0 ns,[11] and *n* = 2.1 was calculated from a simulation of NBD-C_4 in water. The points corresponding to the probes addressed in this work are highlighted with arrows. For more details, see Table S2.

Table S2 - MD simulation data on orientation and H-bonding, and experimental values of fluorescence lifetime for different NBD lipid probes. Unless stated otherwise, the data were obtained at room temperature/298 K.

	Orientation and H-bonding from MD				Fluorescence lifetime		
Probe	Short axis tilt/ º	Average number of water-NBD NO_2 H-bonds/ fluorophore	System	Ref.	Intensity-averaged τ/ns	System	Ref.
NBD-C_n ($n \geq 8$)	130	0.51	POPC	[8]	5.2	POPC	[4]
22-NBD-Chol	130	0.96	POPC	[9]	6.0 [5] , 6.2 [6]	DMPC, 40 ºC [5] or 37 ºC [6]	[5, 6]
C_{12}-NBD-PC	130	0.51	POPC	This work	6.2	POPC	This work
NBD-PSH	100	0.56	DOPC	[3]	5.9	DOPC	[3]
C_6-NBD-PC	100	0.38	POPC	This work	7.0	POPC	This work
N-NBD-PE	70	0.16	POPC	[10]	8.7	Egg-PC	[7]

NBD-C_n: *N*-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]alkyl; 22-NBD-Chol: 22-(*N*-(7-nitrobenz-2-1,3-benzoxadiazol-4-yl)amino)-23,24-bisnor-5-cholen-3β-ol; C_{12}-NBD-PC: 1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-sn-glycero-3-phosphocholine; NBD-PSH: 1,2-dioleoyl-sn-glycero-3-phospho-L-serine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl); C_6-NBD-PC: 1-palmitoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-*sn*-glycero-3-phosphocholine; *N*-NBD-PE: 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl); POPC: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DOPC: 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DMPC: 1,2-dimyristoyl-sn-glycero-3-phosphocholine; Egg-PC: L-α-phosphatidylcholine from chicken egg.

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