Journal Name

ARTICLE

ESI for:

CHARMM force field parameterization protocol for self-assembling peptide amphiphiles: The Fmoc moiety

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

I. Ramos Sasselli,[a] R. V. Ulijn[a,b] and T. Tuttle[a,*]

[a] *Pure and Applied Chemistry Department, WestCHEM. University of Strathclyde. 295 Cathedral Street, Glasgow, G1 1XL, UK.*
[b] *Advanced Science Research Center (ASRC) and Hunter College, City University of New York, 85 St Nicholas Terrace, New York, New York 10031, USA.*
* E-mail: tell.tuttle@strath.ac.uk

Here the topology and parameters information of the Fmoc moiety for the CHARMM force field is included in the NAMD format:

CHARMM TOPOLOGY (NAMD Format)

```
!hydrogens
MASS     1 HGA2     1.00800
MASS     2 HGR61    1.00800
!carbons
MASS     3 CG2O1   12.01100
MASS     4 C2R61   12.01100
MASS     5 CG2R66  12.01100
MASS     6 C2R67   12.01100
MASS     7 C2RC0   12.01100
MASS     8 CG321   12.01100
MASS     9 C3C52   12.01100
!oxygens
MASS    10 OG2D1   15.99940
MASS    11 OG302   15.99940

DEFA FIRS NONE LAST NONE
AUTO ANGLES DIHE

RESI FMO         0.00

GROUP
ATOM C1   C2R61   -0.110 !           H4          H7
ATOM H1   HGR61    0.135 !           |           |
ATOM C2   C2R61   -0.110 !           C4          C7
ATOM H2   HGR61    0.135 !         //  \         /  \\
ATOM C3   C2R61   -0.110 !    H3--C3    C5----C6    C8--H8
ATOM H3   HGR61    0.135 !        |     ||    ||     |
ATOM C4   C2R61   -0.110 !    H2--C2    C13   C11   C9--H9
ATOM H4   HGR61    0.135 !         \\  /  \  /  \  //
ATOM C5   C2R67   -0.100 !           C1    C12   C10
ATOM C6   C2R67   -0.100 !           |    /  \    |
ATOM C7   C2R61   -0.110 !          H1   H21 |  H10
ATOM H7   HGR61    0.135 !                  |
ATOM C8   C2R61   -0.110 !              HF1--CF1--HF2
ATOM H8   HGR61    0.135 !                   |
ATOM C9   C2R61   -0.110 !                  OF1
ATOM H9   HGR61    0.135 !                   |
ATOM C10  C2R61   -0.110 !                  C==OF2
ATOM H10  HGR61    0.135 !                   |
ATOM C11  C2RC0   -0.050 !          TO AMINO ACID RESIDUE NH
ATOM C12  C3C52    0.150
ATOM H21  HGA2     0.090
ATOM C13  C2RC0   -0.050

GROUP
ATOM CF1  CG321    0.240
ATOM HF1  HGA2     0.220
ATOM HF2  HGA2     0.220
ATOM OF1  OG302   -0.920
ATOM C    CG2O1    0.950
ATOM OF2  OG2D1   -0.850

BOND C1  C2  C2  C3  C3  C4  C4  C5  C5  C6
BOND C6  C7  C7  C8  C8  C9  C9  C10 C10 C11
BOND C11 C12 C12 C13 C13 C1  C5  C13 C6  C11
BOND C1  H1  C2  H2  C3  H3  C4  H4  C7  H7
BOND C8  H8  C9  H9  C10 H10 C12 H21 C12 CF1
BOND CF1 HF1 CF1 HF2 CF1 OF1 OF1 C
DOUBLE C   OF2
BOND C   +N
```

```
IC C5   C13  C1    C2    0.0000    0.00    0.00    0.00   0.0000
IC C1   C13  C5    C4    0.0000    0.00    0.00    0.00   0.0000
IC C13  C5   C4    C3    0.0000    0.00    0.00    0.00   0.0000
IC C1   C5   *C13  C12   0.0000    0.00  180.00    0.00   0.0000
IC C4   C13  *C5   C6    0.0000    0.00  180.00    0.00   0.0000
IC C13  C5   C6    C11   0.0000    0.00    5.00    0.00   0.0000
IC C5   C11  *C6   C7    0.0000    0.00  180.00    0.00   0.0000
IC C7   C6   C11   C10   0.0000    0.00    0.00    0.00   0.0000
IC C11  C6   C7    C8    0.0000    0.00    0.00    0.00   0.0000
IC C6   C11  C10   C9    0.0000    0.00    0.00    0.00   0.0000
IC C13  C2   *C1   H1    0.0000    0.00  180.00    0.00   0.0000
IC C1   C3   *C2   H2    0.0000    0.00  180.00    0.00   0.0000
IC C2   C4   *C3   H3    0.0000    0.00  180.00    0.00   0.0000
IC C3   C5   *C4   H4    0.0000    0.00  180.00    0.00   0.0000
IC C6   C8   *C7   H7    0.0000    0.00  180.00    0.00   0.0000
IC C7   C9   *C8   H8    0.0000    0.00  180.00    0.00   0.0000
IC C8   C10  *C9   H9    0.0000    0.00  180.00    0.00   0.0000
IC C9   C11  *C10  H10   0.0000    0.00  180.00    0.00   0.0000
IC C11  C13  *C12  H21   0.0000    0.00  120.00    0.00   0.0000
IC C11  C13  *C12  CF1   0.0000    0.00 -120.00    0.00   0.0000
IC C12  CF1  C11   C10   0.0000    0.00  180.00    0.00   0.0000
IC C12  CF1  C13   C1    0.0000    0.00  180.00    0.00   0.0000
IC CF1  C11  *C12  C13   0.0000    0.00  120.00    0.00   0.0000
IC OF1  CF1  C12   HF1   0.0000    0.00  180.00    0.00   0.0000
IC OF1  HF2  *CF1  C12   0.0000    0.00  180.00    0.00   0.0000
IC OF2  +N   *C    OF2   0.0000    0.00  180.00    0.00   0.0000
IC +N   C    OF1   CF1   0.0000    0.00  180.00    0.00   0.0000
IC C    OF1  CF1   HF1   0.0000    0.00    0.00    0.00   0.0000
IC C    OF1  CF1   HF2   0.0000    0.00  120.00    0.00   0.0000
IC C    OF1  CF1   C12   0.0000    0.00  240.00    0.00   0.0000
```

PARAMETERS (NAMD Format)

BONDS

```
C2R61 HGR61  340.00    1.0800
C2R61 C2R61  305.00    1.3750
C2R61 C2R67  305.00    1.3750
C2R61 C2RC0  300.00    1.3600
C2R67 C2R67  300.00    1.4900
C2R67 C2RC0  300.00    1.4200
C2RC0 C3C52  305.00    1.5200
C3C52 HGA2   307.00    1.1000
CG321 C3C52  195.00    1.5180
CG321 HGA2   309.00    1.1110
CG321 OG302  320.00    1.4400
CG2O1 OG302  340.00    1.4300
CG2O1 OG2D1  620.00    1.2300
CG2O1 NH1    370.00    1.3450
NH1   H      440.00    0.9970
NH1   CT1    320.00    1.4300
CT1   CD     200.00    1.5220
CT2   CT1    222.50    1.5380
HB    CT1    330.00    1.0800
HA    CT2    309.00    1.1110
OH1   CT2    428.00    1.4200
OH1   H      545.00    0.9600
OB    CD     750.00    1.2200
OH1   CD     230.00    1.4000
```

ANGLES

```
CG2O1  OG302  CG321   55.00  109.00
OG2D1  CG2O1  OG302   90.00  125.90
C2RC0  C3C52  CG321   38.00  114.00
C3C52  CG321  OG302   75.70  115.10
C3C52  CG321  HGA2    38.50  115.10
CG321  C3C52  HGA2    38.50  106.80
```

```
NH1     CG2O1   OG302    80.00  116.50
NH1     CG2O1   OG2D1    80.00  122.50
CG2O1   NH1     H        34.00  123.00
C2R61   C2R61   C2R61    40.00  120.00   35.00 2.41620
C2R61   C2R61   C2R67    40.00  120.00
C2R61   C2R61   C2RC0    50.00  120.00
C2R61   C2R61   HGR61    30.00  120.00   22.00 2.15250
C2R67   C2R61   HGR61    30.00  120.00
C2RC0   C2R61   HGR61    30.00  120.00   22.00 2.14600
C2R61   C2R67   C2R67    40.00  120.00
C2R61   C2R67   C2RC0    50.00  120.00
C2R67   C2R67   C2RC0    55.00  110.00
C2R61   C2RC0   C2R67    50.00  120.00
C2R61   C2RC0   C3C52    60.00  130.00
C2R67   C2RC0   C3C52   110.00  110.00
OG302   CG321   HGA2     60.00  109.50
HGA2    CG321   HGA2     35.50  109.00    5.40 1.80200
C2RC0   C3C52   C2RC0    40.00   95.00
C2RC0   C3C52   HGA2     38.00  114.00
HB      CT1     CD       50.00  109.50
H       OH1     CD       55.00  115.00
OB      CD      CT1      70.00  125.00   20.00 2.44200
CT1     CD      OH1      55.00  110.50
OH1     CD      OB       50.00  123.00  210.00 2.26200
NH1     CT1     HB       48.00  108.00
NH1     CT1     CD       50.00  107.00
H       NH1     CT1      35.00  117.00
HA      CT2     CT1      33.43  110.10   22.53 2.17900
OH1     CT2     CT1      75.70  110.10
CG2O1   NH1     CT1      50.00  120.00
H       OH1     CT2      57.50  106.00
HB      CT1     CT2      35.00  111.00
CT2     CT1     CD       52.00  108.00
HA      CT2     HA       35.50  109.00    5.40 1.80200
OH1     CT2     HA       45.90  108.89
NH1     CT1     CT2      70.00  113.50

DIHEDRALS
C2R61   C2RC0   C3C52   CG321   0.5000  3   0.00
C2R67   C2RC0   C3C52   CG321   0.5000  3   0.00
C2RC0   C3C52   CG321   HGA2    0.1950  3   0.00
C2RC0   C3C52   CG321   OG302   0.1950  3   0.00
C3C52   CG321   OG302   CG2O1   0.0000  3   0.00
HGA2    C3C52   CG321   HGA2    0.1400  3   0.00
HGA2    C3C52   CG321   OG302   0.1400  3   0.00
CG321   OG302   CG2O1   OG2D1   0.9000  1   0.00
CG321   OG302   CG2O1   OG2D1   2.8500  2 180.00
HGA2    CG321   OG302   CG2O1   2.0500  2 180.00
OG2D1   CG2O1   C2R61   C2RC0   1.0000  2 180.00
C2R61   C2R61   C2R61   C2R61   3.1000  2 180.00
C2R61   C2R61   C2R61   C2R67   3.1000  2 180.00
C2R61   C2R61   C2R61   C2RC0   3.0000  2 180.00
C2R61   C2R61   C2R61   HGR61   4.2000  2 180.00
C2R67   C2R61   C2R61   HGR61   4.2000  2 180.00
C2RC0   C2R61   C2R61   HGR61   3.0000  2 180.00
HGR61   C2R61   C2R61   HGR61   2.4000  2 180.00
C2R61   C2R61   C2R67   C2R67   3.1000  2 180.00
C2R61   C2R61   C2R67   C2RC0   0.2500  2 180.00
OG301   C2R61   C2R67   C2R61   3.1000  2 180.00
OG301   C2R61   C2R67   C2R67   3.1000  2 180.00
HGR61   C2R61   C2R67   C2R61   4.2000  2 180.00
HGR61   C2R61   C2R67   C2R67   4.2000  2 180.00
C2R61   C2R61   C2RC0   C2R67   0.2500  2 180.00
C2R61   C2R61   C2RC0   C3C52   0.0000  2 180.00
C2R61   C2R61   C2RC0   C2R67   0.2500  2 180.00
HGR61   C2R61   C2RC0   C3C52   0.0000  2 180.00
C2R61   C2R67   C2R67   C2R61   0.8900  2 180.00
C2R61   C2R67   C2R67   C2RC0   2.0000  2 180.00
C2RC0   C2R67   C2R67   C2RC0   1.5000  2 180.00
C2R61   C2R67   C2RC0   C2R61   0.0500  2 180.00
C2R61   C2R67   C2RC0   C3C52   6.7500  2 180.00
C2R67   C2R67   C2RC0   C2R61   3.5000  2 180.00
C2R67   C2R67   C2RC0   C3C52   5.0000  3 180.00
C2R61   C2RC0   C3C52   C2RC0   0.9000  3   0.00
C2R61   C2RC0   C3C52   HGA2    0.5000  3 180.00
C2R67   C2RC0   C3C52   C2RC0   0.7500  3 180.00
```



```
C2R67  C2RC0  C3C52  HGA2    0.5000  3   0.00
HGR61  C2R61  C2R67  C2RC0   3.0000  2 180.00
HGR61  C2R61  C2RC0  C2R67   3.0000  2 180.00
CG321  OG302  CG2O1  NH1     0.6000  1 180.00
CG321  OG302  CG2O1  NH1     2.0000  2 180.00
OG302  CG2O1  NH1    H       2.5000  2 180.00
OG2D1  CG2O1  NH1    H       2.5000  2 180.00
OG302  CG2O1  NH1    CT1     1.6000  1 180.00
OG2D1  CG2O1  NH1    CT1     2.5000  2 180.00
CG2O1  NH1    CT1    CT2     0.0000  1 180.00
CG2O1  NH1    CT1    CD      0.0000  1 180.00
CG2O1  NH1    CT1    HB      0.0000  1 180.00
H      NH1    CT1    CT2     0.0000  1   0.00
H      NH1    CT1    CD      0.0000  1   0.00
HB     CT1    NH1    H       0.0000  1   0.00
H      OH1    CT2    CT1     1.3000  1   0.00
H      OH1    CT2    CT1     0.3000  2   0.00
H      OH1    CT2    CT1     0.4200  3   0.00
X      CT1    CT2    X       0.2000  3   0.00
X      CD     OH1    X       2.0500  2 180.00
X      CT1    CD     X       0.0000  6 180.00
X      CT2    OH1    X       0.1400  3   0.00

IMPROPERS

CG2O1  X      X      OG2D1    120.00  0     0.00
C2R61  X      X      HGR61     15.00  0     0.00
NH1    X      X         H      20.00  0     0.00
OB     X      X        CD     100.00  0     0.00

NONBONDED nbxmod  5 atom cdiel shift vatom vdistance vswitch -
cutnb 14.0 ctofnb 12.0 ctonnb 10.0 eps 1.0 e14fac 1.0 wmin 1.5

HGA2   0.0  -0.0350  1.3400
HGR61  0.0  -0.0300  1.3582
CG2O1  0.0  -0.1100  2.0000
C2R61  0.0  -0.0700  1.9924
C2R67  0.0  -0.0700  1.9924
C2RC0  0.0  -0.0990  1.8600
CG321  0.0  -0.0560  2.0100   0.0 -0.01 1.90
C3C52  0.0  -0.0600  2.0200   0.0 -0.01 1.90
OG2D1  0.0  -0.1200  1.7000   0.0 -0.12 1.40
OG302  0.0  -0.1000  1.6500
H      0.0  -0.0460  0.2245
NH1    0.0  -0.2000  1.8500   0.0 -0.20 1.55
HA     0.0  -0.0220  1.3200
HB     0.0  -0.0220  1.3200
OH1    0.0  -0.1521  1.7700
OB     0.0  -0.1200  1.7000   0.0 -0.12 1.40
CT1    0.0  -0.0200  2.2750   0.0 -0.01 1.90
CT2    0.0  -0.0550  2.1750   0.0 -0.01 1.90
CD     0.0  -0.0700  2.0000

END
```

K_{ow} DETERMINATION

The experimental determination of the Fmoc-S-OMe K_{ow} was carried out using the shake-flask method. The concentrations of Fmoc-S-OMe in octanol and water were determined by fluorescence spectroscopy. The emission at 304 nm was calibrated in both solvents (Figure ESI 1) using samples of known concentrations. Emission was measured with a Jasco FP-6500 spectrofluorometer at 304 nm, using medium response with an excitation bandwidth of 3nm and an emission bandwidth of 3nm. Samples were measured with 1 cm pathlength cuvettes.

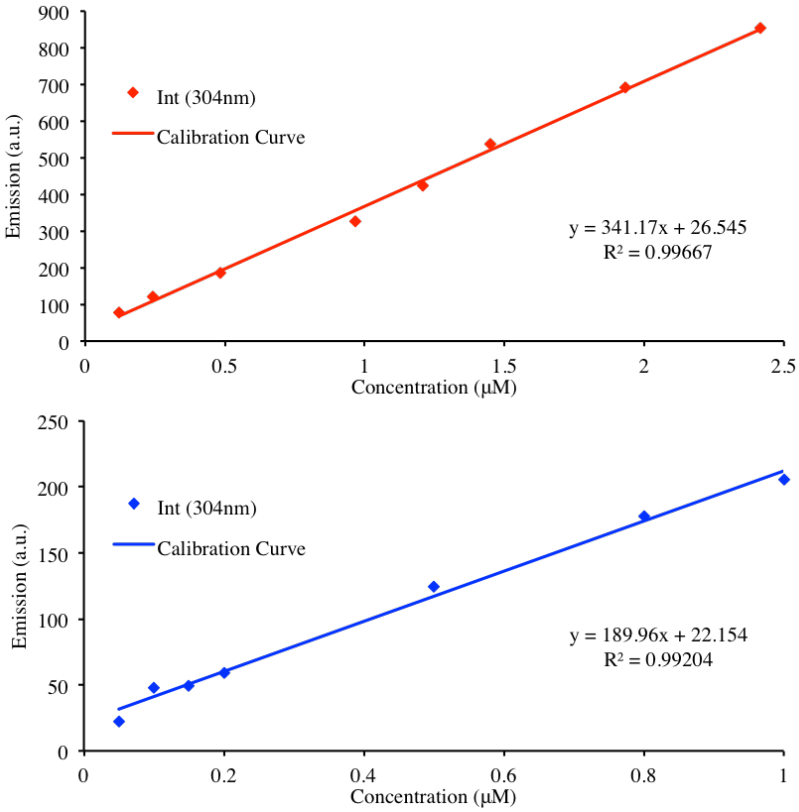

Figure ESI-1. Calibration of the emission intensity of Fmoc-S-OMe in octanol (Red) and in water (Blue) at different concentrations. The range of concentrations was estimated in previous experiments.

Samples for the partition coefficient determination used 2 mL of aqueous Fmoc-S-OMe stock solution (2.01 μM). After mixing them with 2 mL of octanol the mixture was shaken vigorously during 30 s and left resting for 2 hours. 1 mL of each phase was taken to measure fluorescence. Averaged results of 9 samples were used for the final determination of the K_{ow}.

Table ESI-1. Experimental results for the determination of the Fmoc-S-OMe partition coefficient *(K_{ow})*.

	Intensity at 304 nm		*Concentration (μM)*		K_{ow}
	water	octanol	water	octanol	
1	40	706	0.09	1.99	21.1
2	36	692	0.07	1.95	27.3
3	35	713	0.07	2.01	30.2
4	39	704	0.09	1.99	23.2
5	35	705	0.07	1.99	30.5
6	42	770	0.10	2.18	21.7
7	35	678	0.07	1.91	29.9
8	45	701	0.12	1.98	16.8
9	47	726	0.13	2.05	15.7
Average			0.09	2.00	23.6
STD Dev			0.02	0.08	5.5

The final log P or log K_{ow} value is -1.4±0.1. The confidence interval was calculated using equation Eq. ESI. 1:

$$Conf.Interval = \frac{s \cdot t(95\%)}{\sqrt{n}}$$

Where s is the standard deviation, t(95%) is the confidence coefficient for a 95% of confidence level and n is the number of samples used.

Fmoc-S-OMe SOLVATION FREE ENERGIES IN OCTANOL AND IN WATER

The free energy profile as a function of the decoupling of the Fmoc-S-OMe molecule from each solvent is shown in Figure ESI-2. The difference between the starting and the final point gives the solvation free energy of the solute in each solvent (Table ESI-2).

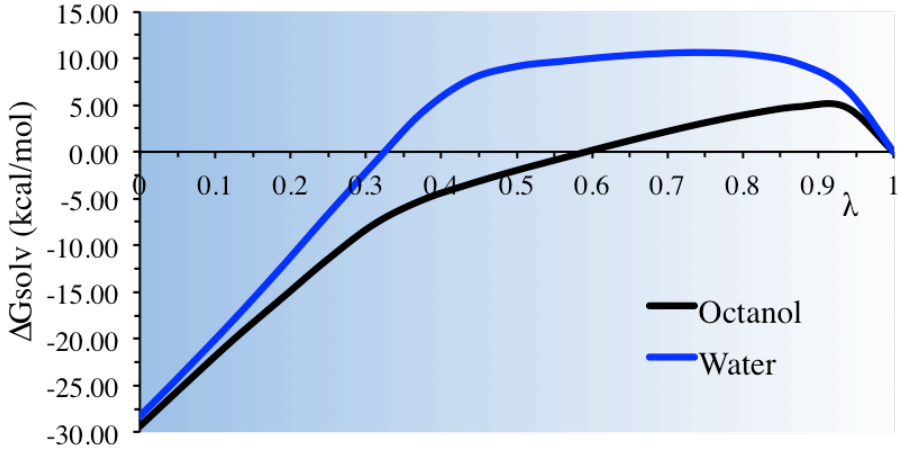

Figure ESI-2. Free energy profiles of the decoupling of Fmoc-S-OH to water and to octanol using MD-FEP.

Table ESI-2. MD-FEP results of the solvation free energies and the final water/octanol partition free energy *(ΔG_{ow})*.

ΔG_{water}	$\Delta G_{octanol}$	$\Delta \mathbf{G_{ow}}$
-28.3 ± 0.1	-29.3 ± 0.3	-1.0 ± 0.2

Fmoc-S-OMe SOLVATION FREE ENERGIES USING FEP: Parameters Study

Prior to the calculation of the final values for the solvation free energies with the FEP different parameters were checked (Table ESI-3). To check the reliability of the results the overlap between the forward and backward simulation was evaluated (Figure ESI-3). It can be seen how the set of FEP parameters used for the calculation of the solvation free energies give the best overlap between both, the forward and the backward, simulations.

Table ESI-3. FEP parameters used in the calculations which results are shown in Figure ESI-3. * The parameters P-4 were used for final results presented in this study.

Parameters Set	*dλ*	*Number of windows*	*Equilibration steps/window*	*Total steps/window*
P-1	0.1	10	2,500	52,500
P-2	0.1	10	30,000	630,000
P-3	0.1	10	500,000	5,500,00
P-4	0.0625	16	250,000	4,250,000

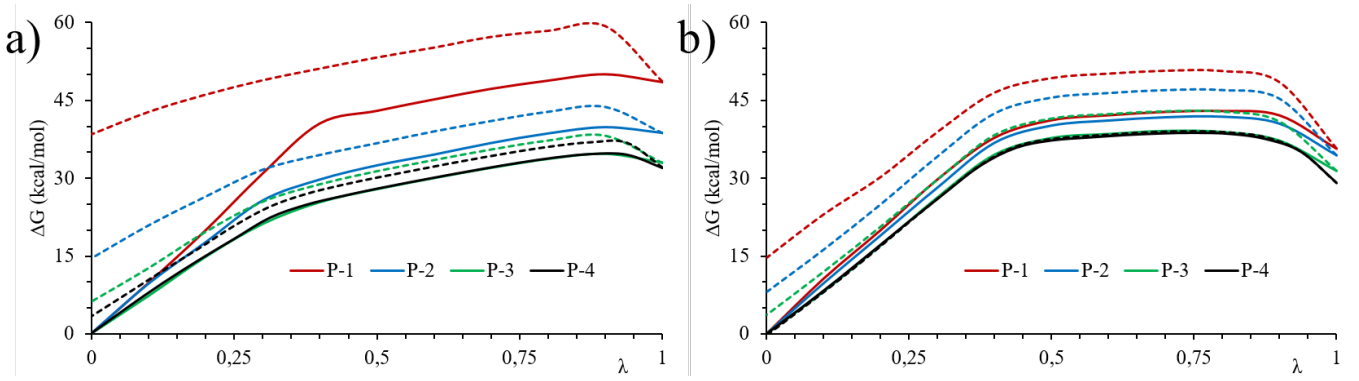

Figure ESI-3. Forward (straight lines) and backward (dashed lines) free energy profiles for the solvation of Fmoc-S-OMe in octanol (a) and in water (b) with the different FEP parameters shown in Table ESI-3.

Fmoc-S-OH PARTICLE SIZE DISTRIBUTION

The AFM image published by Abul-Haija *et al.* was used to carry out the particle size distribution analysis of the Fmoc-S-OH micelles.[1]

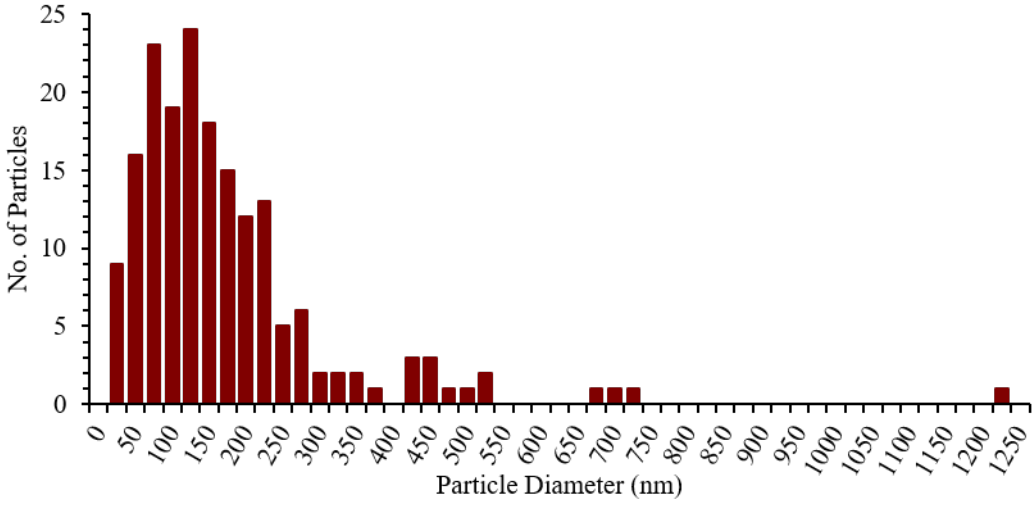

Figure ESI-4. Particle size distribution analysis made on Fmoc-S-OH AFM image from ref. 1.

Fmoc-S-OH MD SIMULATION

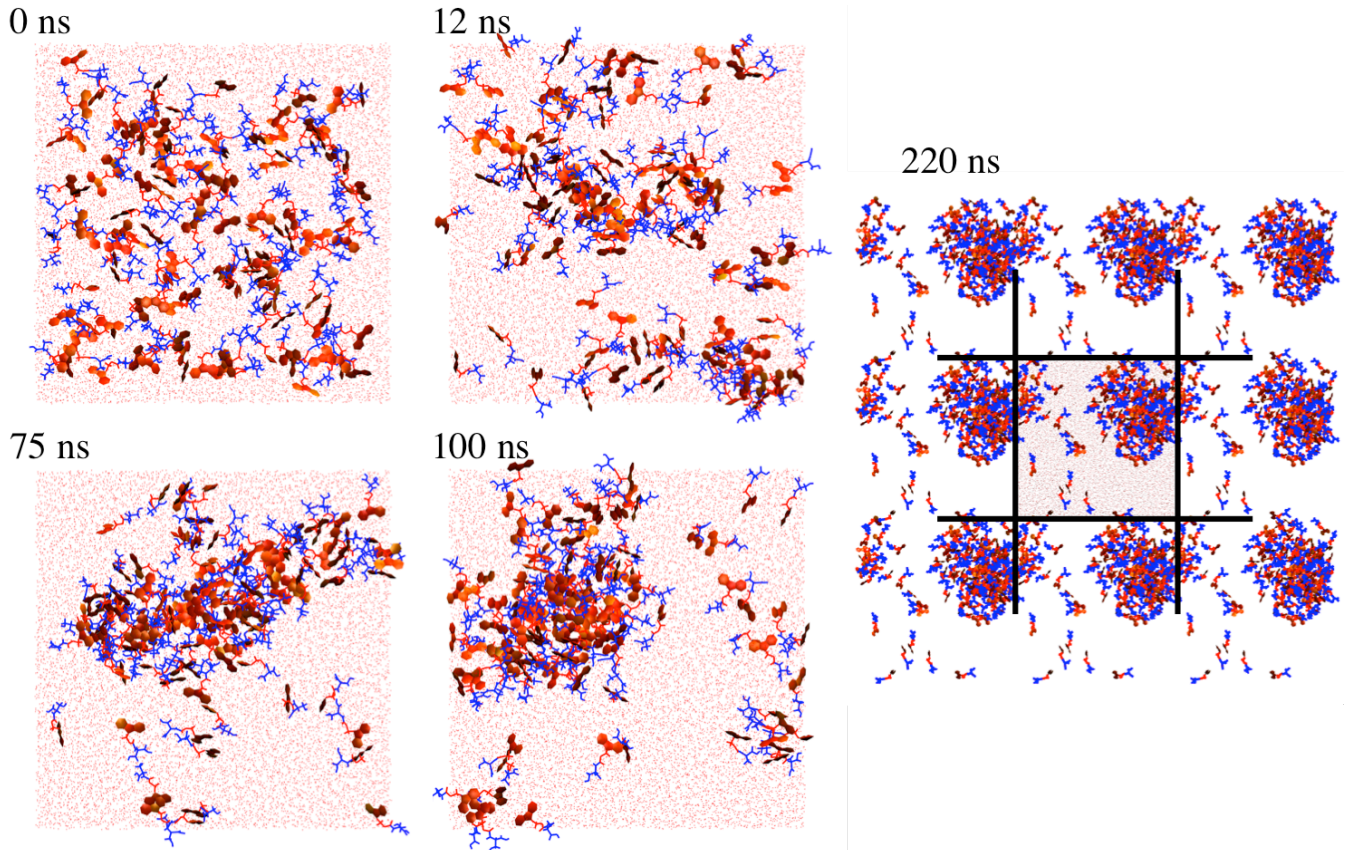

Figure ESI-5. Snapshots of the Fmoc-S-OH simulation (Fmoc in red and S-OH in blue). The last snapshot (220 ns) shows also the periodic images in the *xy*-plane for clarity.

Fmoc-Y-OH MD SIMULATION

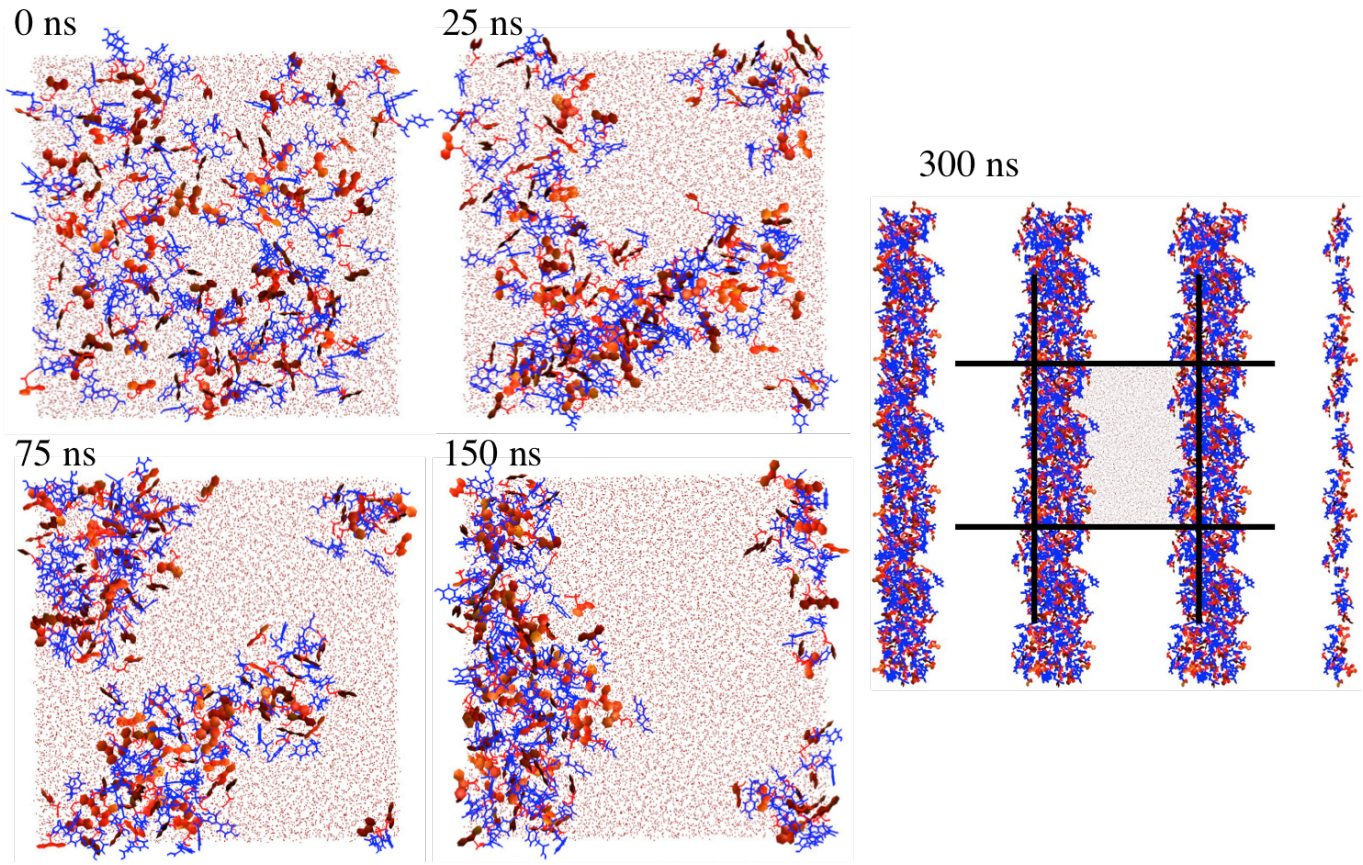

Figure ESI-6. Snapshots of the Fmoc-Y-OH simulation (Fmoc in red and Y-OH in blue). The last snapshot (300 ns) shows also the periodic images in the *xy*-plane for clarity.

1. Y. M. Abul-Haija, S. Roy, P. W. Frederix, N. Javid, V. Jayawarna and R. V. Ulijn, *Small*, 2014, **10**, 973-979.