Electronic Supplementary Material (ESI) for RSC Advances.

Copper-free click chemistry for microdroplet's W/O interface engineering.

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1 Chemical synthesis

1.1 Material and Methods

Experimental procedures.
Unless otherwise indicated, reactions were carried out under an argon atmosphere in flame-dried glassware with magnetic stirring. Air and/or moisture-sensitive liquids were transferred *via* syringe. When required, solutions were degassed by argon bubbling through a needle. Organic solutions were concentrated by rotary evaporation at 25-80 °C at 15-30 torr. Analytical thin layer chromatography (TLC) was performed using plates cut from aluminium sheets (ALUGRAM Xtra SIL G/UV_{254} from Macherey-Nagel). Visualization was achieved under a 254 or 365 nm UV light and by immersion in an appropriate revelation solution.

Materials.
All reagents were obtained from commercial sources and used without any further purifications. Anhydrous solvents used in experiments were obtained from Sigma-Aldrich or Alfa Aesar. Fluorinated solvents (HFE 7100, HFE 7500 and FC 3283) were purchased from 3M. Krytox157FS(H) was purchased from Dupond. Silica gel for column chromatography was purchased from Merck (Geduran® Si 60, 40-63 µm). Column flash chromatography was carried out using silica gel G-25 (40-63 µm) from Macherey-Nagel.

Instrumentation

NMR spectroscopy, ^{1}H and ^{13}C NMR spectra were recorded respectively at 400 MHz and 100 MHz with a Bruker 400 spectrometer at 23 °C.. ^{19}F NMR spectra were recorded at 375 MHz at with a Bruker 400 spectrometer.Chemical shifts are reported in parts per million (δ) and calibrated using residual non-deuterated solvent. ^{19}F spectra were externally referenced to C_6F_6. Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad or a combination of the above), coupling constant (*J*, Hz) and integration.

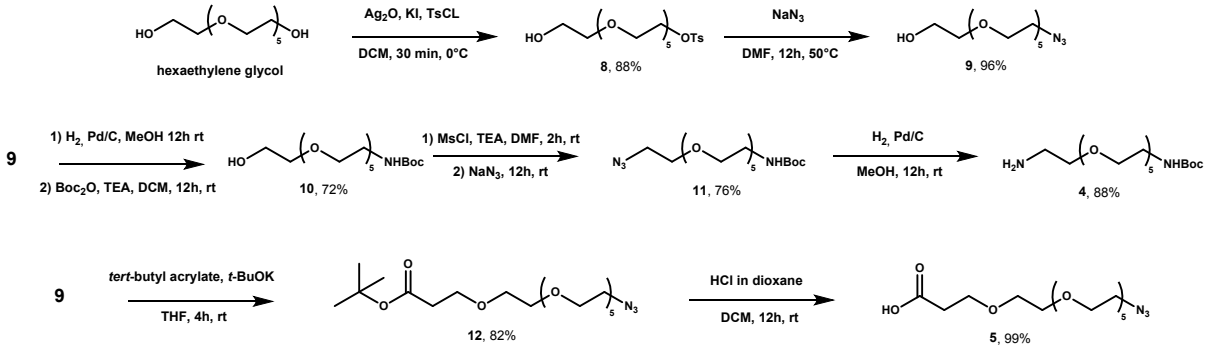
High resolution mass spectra (HRMS) were obtained using a Agilent Q-TOF (time of flight) 6520 and low resolution mass spectra using a Agilent MSD 1200 SL (ESI/APCI) with a Agilent HPLC1200 SL.

Low resolution mass spectra were obtained using a Agilent MSD 1200 SL (ESI/APCI) with a Agilent HPLC1200 SL and a Waters Acquity QDa (ESI) with a Waters Alliance 2695 HPLC.

Preparative HPLC procedures were performed on semi-preparative HPLC Shimadzu Auto-injector SIL-10A (pump: Shimadzu LC-8A, UV-Vis detector: Shimadzu SPD-10A, collector: Shimadzu fraction collector FRC-10A) using a Sunfire C18 (150 mm × 19 mm i.d., 5 µm, Waters) at a flow of 17 mL/min. Per sample 1 mL was injected and water/ACN containing 0.05% TFA was used as eluent system. The gradient applied

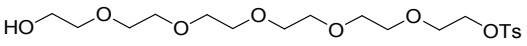
was 5% to 95% ACN in 40 minutes and 10 minutes of re-equilibration. Detection was done at 550 nm for TAMRA derivatives.

1.2 Synthesis of bifunctional peg$_6$ linkers 4 and 5



Scheme S1 : Synthesis of bifunctional peg linkers 4 and 5

17-{[(4-methylphenyl)sulfonyl]oxy}-3,6,9,12,15-pentaoxaheptadecan-1-ol,[1] 8

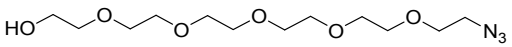


$C_{19}H_{32}O_9S$
MW = 436.52 g/mol

To a solution of hexaethylene glycol (1 eq., 12.1 g, 42.9 mmol) in DCM (380 mL) at 0°C were added KI (0.2 eq., 1.42 g, 0.949 mL, 8.6 mmol) and Ag_2O (1.5 eq., 14.9 g, 64.3 mmol). Tosyl chloride (1.05 eq., 8.58 g, 45.0 mmol) was then added by portion and the reaction mixture was stirred at 0°C for 30 minutes. The mixture was filtered through a pad of celite and concentrated. The crude material was purified by silica gel flash chromatography (DCM to DCM/MeOH 95:5) to give **8** (16.4 g, 37.6 mmol, 88 %) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.77 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 4.14 (t, *J* = 4.8 Hz, 2H), 3.72–3.53 (m, 22H), 2.42 (s, 3H), the OH signal is missing. **^{13}C NMR (100 MHz, $CDCl_3$)**: *δ* 144.8, 133.1, 129.8 (2C), 128.0 (2C), 72.6, 70.7-70.3, 69.3, 68.7, 61.7, 21.6.

17-azido-3,6,9,12,15-pentaoxaheptadecan-1-ol,[2] 9



$C_{12}H_{25}N_3O_6$
MW = 307.34 g/mol

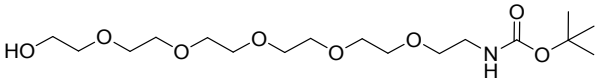
To a mixture of **8** (1 eq., 9.1 g, 20.9 mmol) in 25 mL of DMF was added NaN_3 (1.5 eq., 2.03 g, 31.3 mmol). The reaction was stirred at 50°C for 5 hours and filtered through a pad of celite. After evaporation, 200 mL of DCM were added and the solution was washed with

[1] A. Bouzide and G. Sauve, *Org Lett.*, 2002, **4**, 2329.
[2] M. K. Müller and L. Brunsveld, *Angew. Chem. Int. Ed.*, 2009, **48**, 2921.

brine (3 x 150 mL). The organic layer was dried over $MgSO_4$ and concentrated. The crude material was purified by silica gel flash chromatography (EtOAc to EtOAc/MeOH 9:1 in 25 min) to afford **9** (6.16 g, 20.04 mmol, 96 %) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 3.74–3.47 (m, 22H), 3.32 (d, *J* = 5.0 Hz, 2H), 2.77 (t, *J* = 6.1 Hz, 1H). **^{13}C NMR (100 MHz, $CDCl_3$)**: *δ* 72.5, 70.7–70.6, 70.4, 70.0, 61.8, 50.7.

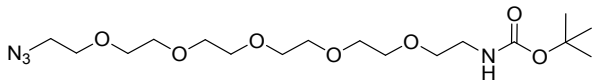
tert-butyl (17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)carbamate, 10



$C_{17}H_{35}NO_8$
MW = 381.46 g/mol

To a solution of **9** (1 eq., 6.80 g, 17.83 mmol) in MeOH (250 mL) was added Pd/C (2 %, 0.471 g, 0.44 mmol) and the reaction mixture was stirred at room temperature under atmospheric pressure of hydrogen. After 12 hours, the mixture was filtered through a pad of celite and concentrated. The crude material was dissolved in DCM and a solution of Boc_2O (1.2 eq., 5.77 g, 5.66 mL, 26.4 mmol) and TEA (2 eq., 4.46 g, 6.13 mL, 44.1 mmol) in DCM (50 mL) was added. The reaction mixture was stirred at room temperature for 12 hours. 200 mL of water were added and the mixture was extracted with DCM (4 x 150 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude was purified by flash chromatography (EtOAc 5 min then DCM to DCM/MeOH 90:10 in 30 min) to afford *tert*-butyl (17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)carbamate **10** (6.05 g, 15.9 mmol, 72 %) as a yellowish oil.
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 5.13 (brs, 1H), 3.85–3.55 (m, 20H), 3.49 (dt, *J* = 5.8, 3.9 Hz, 2H), 3.29 (d, *J* = 4.9 Hz, 2H), 2.74 (brs, 1H), 1.42 (s, 9H). **^{13}C NMR (100 MHz, $CDCl_3$)**: *δ* 156.0, 79.1, 77.4, 77.2, 77.0, 76.7, 72.6, 70.6–70.5, 70.4, 70.3, 61.7, 40.4, 28.4 (3C).

tert-butyl *N*-(17-azido-3,6,9,12,15-pentaoxaheptadecyl)carbamate, 11

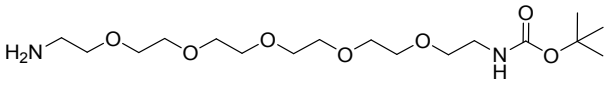


$C_{17}H_{34}N_4O_7$
MW = 406.47 g/mol

To a solution of *tert*-butyl (17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)carbamate **10** (1 eq., 6 g, 15.7 mmol) and TEA (5 eq., 7.96 g, 10.9 mL, 78.6 mmol) in DMF (30 mL) under argon at 0°C was added MsCl (2 eq., 3.6 g, 2.43 mL, 31.5 mmol). The mixture was stirred at room temperature for 2 hours then NaN_3 (2.2 eq., 2.25 g, 1.22 mL, 34.6 mmol) was added. The reaction was stirred at room temperature for 12 hours. After concentration, 150 mL of water were added and the mixture was extracted with DCM (3 x 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude was purified by flash chromatography (DCM to DCM/MeOH 9:1 in 30 min) to afford *tert*-butyl *N*-(17-azido-3,6,9,12,15-pentaoxaheptadecan-1-yl)carbamate **11** (4.86 g, 12 mmol, 76 %) as a yellowish oil.

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 5.03 (s, 1H), 3.74–3.58 (m, 18H), 3.53 (t, *J* = 5.1 Hz, 2H), 3.38 (t, *J* = 5.1 Hz, 2H), 3.35–3.26 (m, 2H), 1.44 (s, 9H). **^{13}C NMR (100 MHz, $CDCl_3$)**: *δ* 156.0, 79.2, 77.3, 77.2, 77.0, 76.7, 70.7–70.6, 70.3, 70.3, 70.1, 50.7, 40.4, 28.5 (3C).

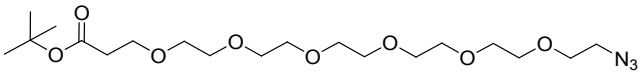
tert-butyl *N*-(17-amino-3,6,9,12,15-pentaoxaheptadecyl)carbamate, 4[3]



$C_{17}H_{36}N_2O_7$
MW = 380.48 g/mol

tert-butyl *N*-(17-azido-3,6,9,12,15-pentaoxaheptadecan-1-yl)carbamate **11** (1 eq., 2.6 g, 6.4 mmol) was dissolved in MeOH (243 mL). Pd/C (2 %, 0.136 g, 0.128 mmol) was added and the mixture was stirred at 0°C under atmospheric pressure of H_2 for 24 hours. The mixture was filtered through celite and concentrated *in vacuo*. The crude was purified by flash chromatography (DCM to DCM/MeOH (10% NH_4OH) 9:1) to afford *tert*-butyl *N*-(17-amino-3,6,9,12,15-pentaoxaheptadecan-1-yl)carbamate **4** (2.15 g, 5.65 mmol, 88 %) as a yellowish oil.
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 5.14 (s, 1H), 3.75–3.58 (m, 16H), 3.57–3.45 (m, 4H), 3.37–3.21 (m, 2H), 2.86 (t, *J* = 5.2 Hz, 2H), 1.44 (s, 9H). The NH_2 signal is missing. **^{13}C NMR (100 MHz, $CDCl_3$)**: *δ* 155.8, 78.4, 71.8, 70.2–70.1, 69.9, 41.0, 40.0, 28.2 (3C).

tert-butyl 1-azido-3,6,9,12,15,18-hexaoxahenicosan-21-oate, 12



$C_{19}H_{37}N_3O_8$
MW = 435.51 g/mol

To a solution of 17-azido-3,6,9,12,15-pentaoxaheptadecan-1-ol **9** (1 eq., 3.7 g, 12 mmol) and *t*-BuOK (0.1 eq., 0.135 g, 1.2 mmol) in THF (69.4 mL) at 0°C was added *tert*-butyl acrylate (1.3 eq., 2 g, 2.27 mL, 15.6 mmol). The mixture was stirred at room temperature for 14 hours. After evaporation, 75 mL of water were added and the mixture was extracted with EtOAc (4 x 75 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude material was purified by silica gel flash chromatography (cyclohexane/EtOAc 8:2 to EtOAc in 30 minutes) to afford *tert*-butyl 1-azido-3,6,9,12,15,18-hexaoxahenicosan-21-oate (4.28 g, 9.82 mmol, 82 %) **12** as a yellowish oil.

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 3.83–3.51 (m, 24H), 3.38 (t, *J* = 5.1 Hz, 2H), 2.50 (t, *J* = 6.6 Hz, 2H), 1.45 (s, 9H). **^{13}C NMR (100 MHz, $CDCl_3$)**: *δ* 170.9, 80.5, 77.3, 77.0, 76.7, 70.7–70.5, 70.4, 70.0, 66.9, 53.4, 50.7, 36.3, 28.1 (3C).

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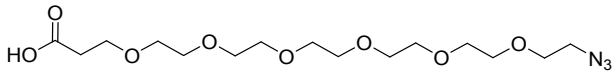
[3] J. G. A. Walton, S. Patterson, G. Liu, J. D. Haraldsen, J. J. Hollick, A. M. Z. Slawin, G. E. Ward and N. J. Westwood, *Org. Biomol. Chem.*, 2009, **7**, 3049.

1-azido-3,6,9,12,15,18-hexaoxahenicosan-21-oic acid, 5

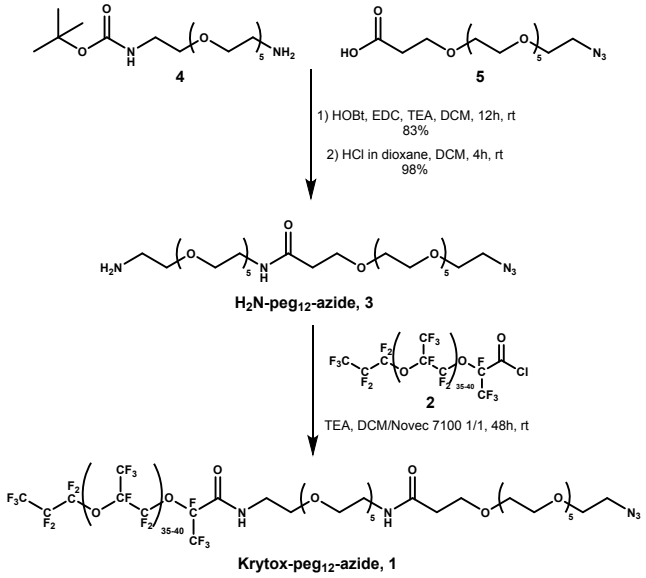


$C_{15}H_{29}N_3O_8$
MW = 379.41 g/mol

*t*ert-Butyl 1-azido-3,6,9,12,15,18-hexaoxahenicosan-21-oate **12** (1 eq., 2.8 g, 6.43 mmol) was dissolved in DCM (30.5 mL). A solution of HCl in dioxane (15 eq., 4 M, 24.1 mL, 96.4 mmol) was added dropwise and the reaction was stirred at room temperature for 12 hours. After concentration, 1-azido-3,6,9,12,15,18-hexaoxahenicosan-21-oic acid **5** (2.41 g, 6.36 mmol, 99 %) as a brown oil.

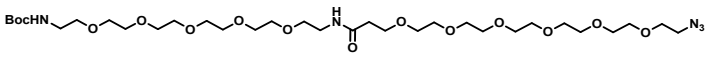
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 3.77 (t, *J* = 6.1 Hz, 2H), 3.70–3.61 (m, 22H), 3.39 (t, *J* = 5.0 Hz, 2H), 2.61 (t, *J* = 6.1 Hz, 2H). The CO_2H signal is missing. **^{13}C NMR (100 MHz, $CDCl_3$)**: *δ* 174.8, 77.4, 77.3, 77.0, 76.7, 70.7–70.5, 70.5, 70.3, 70.0, 66.5, 50.7, 35.0.

1.3 Synthesis of azide fluorosurfactant, Krytox-peg$_{12}$-N$_3$ 1



Scheme S2 : Synthesis of Krytox-peg$_{12}$-azide

BocNH-peg$_{12}$-azide, 13
(*tert*-Butyl (39-azido-19-oxo-3,6,9,12,15,22,25,28,31,34,37-undecaoxa-18-azanonatriacontyl)carbamate)



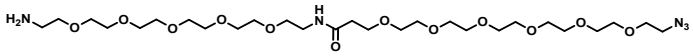
$C_{32}H_{63}N_5O_{14}$
MW = 741.87 g/mol

To a solution of 1-azido-3,6,9,12,15,18-hexaoxahenicosan-21-oic acid **5** (1 eq., 1.00 g, 2.64 mmol) in $CHCl_3$ (15 mL) were added 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (1.5 eq., 0.76 g, 3.95 mmol) and HOBt (1.5 eq., 0.53 g, 3.95 mmol). The resulting mixture was stirred at room temperature for 20 minutes. A solution of *tert*-butyl *N*-(17-amino-3,6,9,12,15-pentaoxaheptadecan-1-yl)carbamate **4** (1.2 eq., 1.20 g, 3.16 mmol) and DIEA (2.5 eq., 1.09 mL, 6.59 mmol) in $CHCl_3$ (10 mL) was added and the reaction was stirred at room temperature for 14 hours. The resulting solution was diluted with water (50 mL) and extracted with DCM (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (25 g; DCM to DCM/MeOH 95:5 in 30 minutes) afforded BocNH-peg_{12}-azide **13** (1.62 g, 2.19 mmol, 83 %) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.62 (brs, 1H), 5.05 (brs, 1H), 3.74 (t, J = 6.0 Hz, 2H), 3.70–3.58 (m, 38H), 3.54 (q, J = 5.4 Hz, 4H), 3.44 (dd, J = 5.4 and 10.8 Hz, 2H), 3.39 (d, J = 5.0 Hz, 2H), 3.31 (dd, J = 5.0 and 10.0 Hz, 2H), 2.47 (t, J = 6.0 Hz, 2H), 1.44 (s, 9H). **^{13}C NMR (100 MHz, $CDCl_3$):** δ 171.2, 155.8, 78.7, 70.5–69.7, 67.1, 50.5, 40.2, 39.0, 36.8, 28.3 (3C). **MS (ESI) m/z:** 764.2 $[M + Na]^+$.

H_2N-peg_{12}-azide, 3

(*N*-(17-Amino-3,6,9,12,15-pentaoxaheptadecyl)-1-azido-3,6,9,12,15,18-hexaoxahenicosan-21-amide)



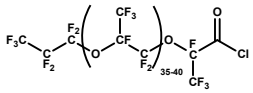
$C_{27}H_{55}N_5O_{12}$

MW = 641.75 g/mol

To a solution of BocNH-peg_{12}-azide **13** (1 eq., 0.40 g, 0.54 mmol) in DCM (15 mL) was added a 4M solution of HCl in dioxane (15 eq., 2.02 mL, 8.09 mmol) and the reaction mixture was stirred at room temperature for 4 hours. After evaporation the crude product was purified by flash chromatography (15 g, DCM to DCM/MeOH/NH_4OH 9:1.8:0.2) to afford NH_2-peg_{12}-azide **3** (0.34 g, 0.53 mmol, 98 %) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (brs, 1H), 3.82–3.56 (m, 46H), 3.44 (dd, J = 5.3 and 10.5 Hz, 2H), 3.38 (t, J = 5.0 Hz, 2H), 3.01 (t, J = 5.0 Hz, 2H), 2.55 (t, J = 6.2 Hz, 2H). **^{13}C NMR (100 MHz, $CDCl_3$):** δ 171.4, 70.4–70.0, 69.7, 69.6, 69.5, 69.0, 67.1, 50.4, 40.4, 38.8, 36.5. **MS (ESI) m/z:** 664.3 $[M + Na]^+$.

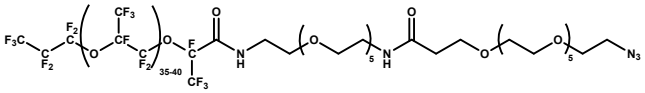
Krytox-COCl, 2



To a solution of Krytox-157FSH-CO_2H (5 g) in 40 mL of HFE7100 were added 3.4 mL of oxalyl chloride and the reaction mixture was stirred and refluxed at 65 °C under argon for 24 hours. After cooling to room temperature, the mixture was filtered through paper and concentrated. The obtained Krytox acid chloride was used without further purification.

^{19}F NMR (375 MHz, C_6D_6/C_6F_6): *δ* -79.16 – -81.37 (m, 175F), -122.98 – -126.63 (m, 1F), -130.39 (s, 2F), -144.22 – -145.38 (m, 35F).

Krytox-peg$_{12}$-azide, 1



To a solution of Krytox157FSH-COCl **2** (2.30 g) in HFE 7100 (15 mL) was added dropwise a solution of NH_2-peg$_{12}$-azide **3** (0.25 g) and TEA (148 µL) in DCM (15 mL). The resulting mixture was vigorously stirred at room temperature for 36 hours. The crude material obtained after evaporation of the solvent was dissolved in FC 3283 (150 mL). The resulting solution was transferred in a separatory funnel and DCM (100 mL) was added forming an emulsion. The fluorinated layer was concentrated under reduced pressure to afford Krytox-peg$_{12}$-azide **1** as sticky oil.

^{19}F NMR (375 MHz, C_6D_6/C_6F_6): *δ* -78.87 – -81.26 (m), -126.08 – -127.04 (m, 0.80F, amide product), -130.41 (s, 2F), -131.96 – -133.00 (m, 0.20F, PFPE carboxylate), -144.05 – -145.42 (m).

1.4 ^{19}F NMR characterization of azide fluorosurfactant

The conversion of the Krytox carboxylic acid group to the non-ionic amide derivative was investigated by ^{19}F NMR spectroscopy as reported by Holtze *et al.*[4](Figure S1). By monitoring the chemical shift of the fluorine atom in α position of the carbonyl group, the PFPE amide/PFPE carboxylate ratio can be determined (Figure S2). To support this NMR attribution we prepared the PFPE carboxylate by mixing Krytox-CO_2H and triethylamine, and characterized it by ^{19}F NMR in the same condition. An overlay comprising NMR spectra of Krytox-acyl chloride **2**, Krytox-carboxylate salt and Krytox-peg$_{12}$-azide **1** has been also added (Figure S3).

[4] C. Holtze, A. C. Rowat, J. J. Agresti, J. B. Hutchison, F. E. Angilè, C. H. J. Schmitz, S. Köster, H. Duan, K. J. Humphry, R. A. Scanga, J. S. Johnson, D. Pisignano and D. A. Weitz, *Lab Chip*, 2008, **8**, 1632.

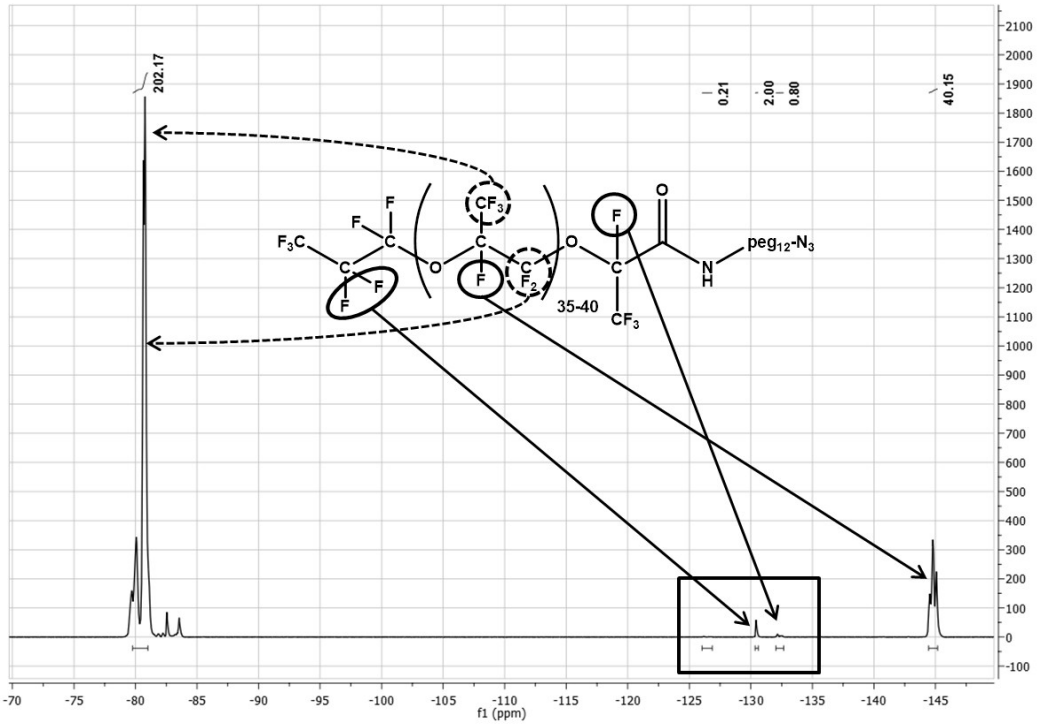


Figure S1 : ^{19}F NMR characterization of Krytox-peg$_{12}$-N$_3$ (1). Attribution of signals according to the results reported by Holtze *et al.*[4]

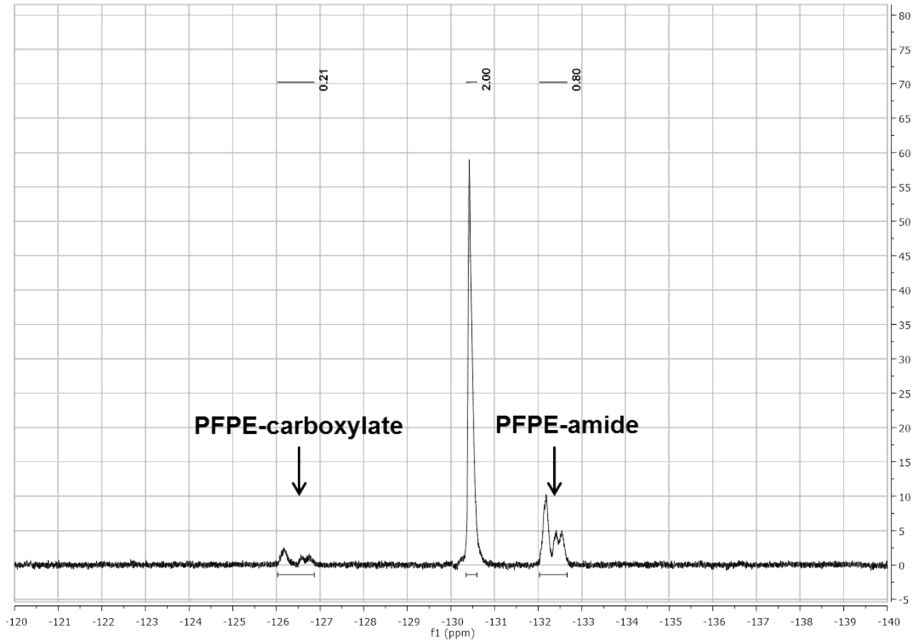


Figure S2 : ^{19}F NMR characterization of Krytox-peg$_{12}$-N$_3$ (1). Zoom:-140 to -120 ppm, chemical shift of fluorine in α position of the carbonyl group.

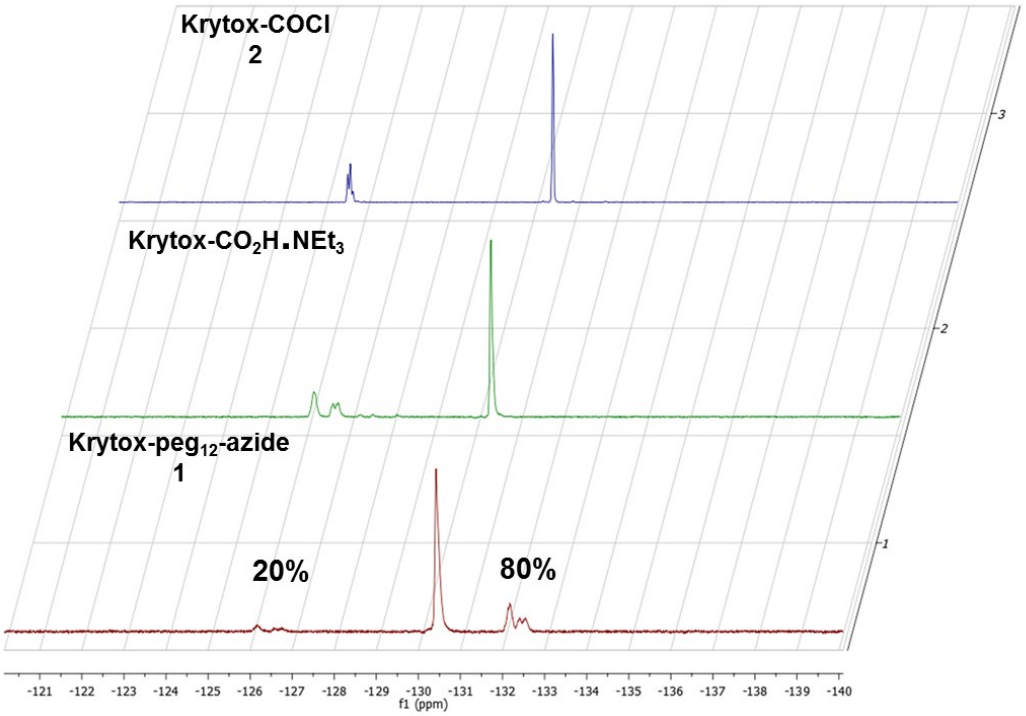


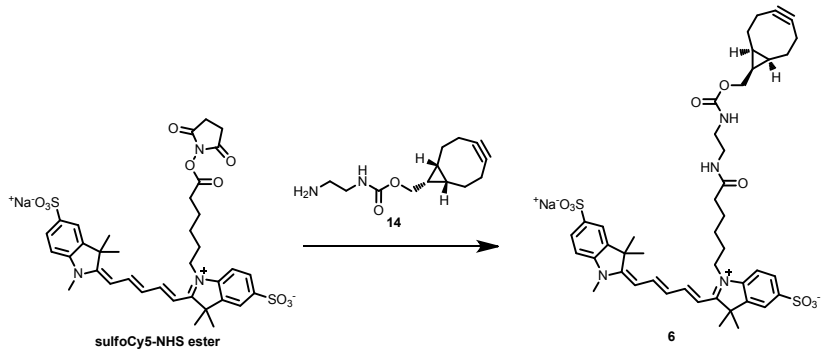
Figure S3 : ^{19}F characterization of KrytoxCOCl (2), KrytoxCO_2H-triethylamine salt and Krytox-peg$_{12}$-azide (1). Zoom -140 to -120 ppm.

1.5 Synthesis of fluorescent strained cycloalkyne derivatives

To establish a proof of concept of the capture at the inner surface of the microdroplet by copper-free click chemistry, two fluorescent strained cycloalkyne derivatives have been synthesized. BCN ((1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethyl) was conjugated to TAMRA and sulfo-Cy5 fluorophores. SulfoCy5 NHS ester was directly coupled to BCN-NH_2 **14** *via* an amide bond (Scheme S3). For TAMRA derivative a pseudo-peptidic coupling reaction was first performed between TAMRA-6-CO_2H and mono *N*-boc peg$_6$ diamine derivative **4**. After *N*-boc deprotection, the fluorophore was coupled to BCN in its nitro-phenyl carbonate activated form **15** (Figure S4). (1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate **15**[5] and (1*R*,8*S*,9*S*)-bicyclo[6.1.0]non-4-yn-9-ylmethyl (2-aminoethyl)carbamate **14**[6] were synthesized according to procedures described in the literature.

[5] J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl and F. L. van Delft, *Angew. Chem. Int. Ed.*, 2010, **49**, 9422.

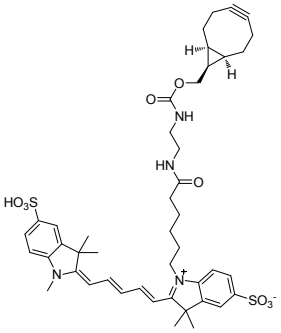
[6] K. Wang, A. Sachdeva, D. J. Cox, N. M. Wilf, K. Lang, S. Wallace, R. A. Mehl and J. W. Chin, *Nat. Chem.*, 2014, **6**, 393.



Scheme S3 : Synthesis of sulfoCy5-BCN click probe

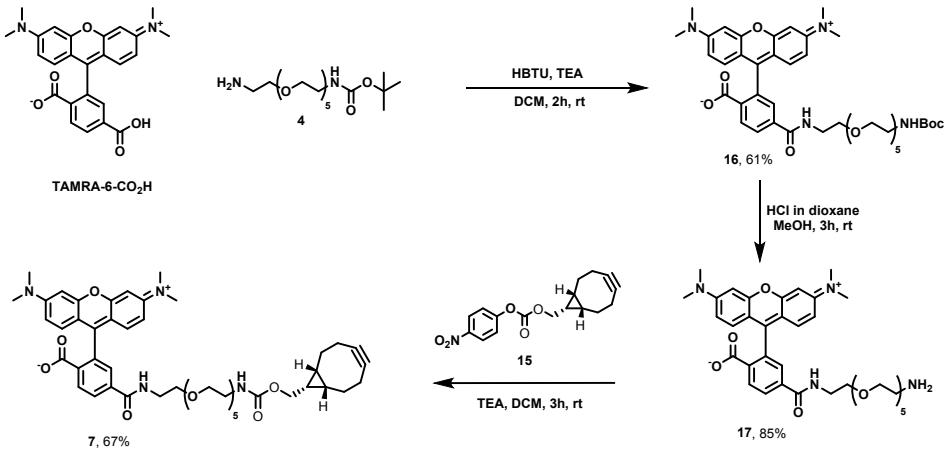
sulfoCy5-BCN 6

1-(6-((2-((((1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethoxy)carbonyl)amino)ethyl)amino)-6-oxohexyl)-3,3-dimethyl-2-((1*E*,3*E*,5*E*)-5-(1,3,3-trimethyl-5-sulfoindolin-2-ylidene)penta-1,3-dien-1-yl)-3*H*-indol-1-ium-5-sulfonate



$C_{45}H_{56}N_4O_9S_2$
MW = 861.08 g/mol

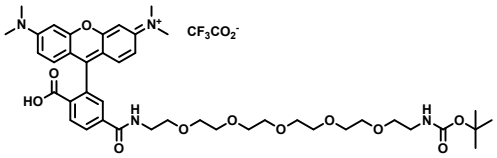
To a solution of sulfoCy5-NHS (1 eq., 8.60 mg, 0.0113 mmol) and DIEA (3 eq., 0.0056 mL, 0.0339 mmol) in DMF (2 mL) was added (1*R*,8*S*,9*S*)-bicyclo[6.1.0]non-4-yn-9-ylmethyl (2-aminoethyl)carbamate **14** (1.2 eq., 3.2 mg, 0.0135 mmol). The reaction was stirred 3 hours at room temperature. After concentration under reduced pressure, the crude material was purified by flash chromatography (RP C18 5 g, H_2O to ACN, 30 minutes) to afford sulfoCy5-BCN **6** (7.70 mg, 0.0087 mmol, 77 %) as a dark solid.
MS (ESI) m/z: 859.3 $[M - H]^-$.



Scheme S4 : Synthesis of TAMRA-peg$_6$-BCN click probe

TAMRA-peg$_6$-NHBoc (TFA salt), 16

(*N*-(9-(2-Carboxy-5-((2,2-dimethyl-4-oxo-3,8,11,14,17,20-hexaoxa-5-azadocosan-22-yl)carbamoyl)phenyl)-6-(dimethylamino)-3*H*-xanthen-3-ylidene)-*N*-methylmethanaminium 2,2,2-trifluoroacetate)



$C_{44}H_{57}F_3N_4O_{13}$

MW = 906.94 g/mol

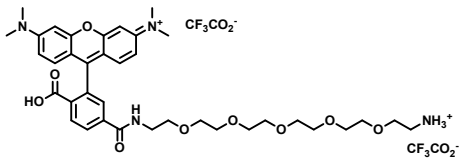
To a solution of TAMRA-6-COOH (1 eq., 60.0 mg, 0.14 mmol) and TEA (3.3 eq., 0.06 mL, 0.46 mmol) in DMF (1 mL) cooled to 0 °C was added HBTU (1.5 eq., 79.3 mg, 0.21 mmol). After 5 minutes a solution of *tert*-butyl *N*-(17-amino-3,6,9,12,15-pentaoxaheptadecan-1-yl)carbamate **4** (1.5 eq., 79.6 mg, 0.209 mmol) in DMF (1 mL) was added and the mixture was stirred for 2 hours at room temperature. Water was added (5 mL) and the mixture was concentrated under reduced pressure. The residue was dissolved in a minimum of MeOH and purified by flash chromatography (RP 16 g, H_2O (0.05% TFA) to ACN, 30 minutes) to afford TAMRA-peg$_6$-NHBoc **16** (67.9 mg, 0.0856 mmol, 61 %) as a pink solid.

^{1}H NMR (400 MHz, CD_3OD): δ 8.39 (d, J = 8.2 Hz, 1H), 8.21 (d, J = 7.0 Hz, 1H), 7.83 (brs, 1H), 7.12 (d, J = 9.5 Hz, 2H), 7.01 (dd, J = 2.0 and 9.4 Hz, 2H), 6.92 (d, J = 1.9 Hz, 2H), 3.78–3.49 (m, 20H), 3.47–3.42 (m, 2H), 3.27 (s, 12H), 3.16 (t, J = 5.5 Hz, 2H), 1.40 (s, 9H), CO_2H and NH signals are missing.

MS (ESI) m/z: 793.4 $[M]^+$.

TAMRA-peg$_6$-NH$_2$ (TFA salt), 28

(1-(4-Carboxy-3-(6-(dimethylamino)-3-(dimethyliminio)-3*H*-xanthen-9-yl)phenyl)-1-oxo-5,8,11,14,17-pentaoxa-2-azanonadecan-19-aminium 2,2,2-trifluoroacetate)



$C_{41}H_{50}F_6N_4O_{13}$

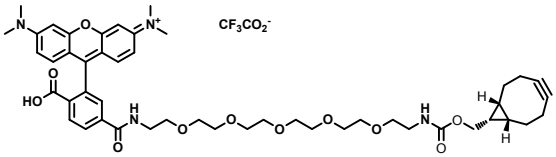
MW = 920.84 g/mol

To a solution of TAMRA-peg$_6$-NHBoc **16** (1 eq., 60 mg, 0.076 mmol) in MeOH (3 mL) was added a 4M solution of HCl in dioxane (15 eq., 0.28 mL, 1.14 mmol) and the reaction was stirred at room temperature for 3 hours. After concentration under reduced pressure, the mixture was dissolved in a minimum of MeOH and purified by flash chromatography (RP 16 g, H_2O (0.05% TFA) to ACN, 30 minutes) to afford TAMRA-peg$_6$-NH$_2$ (TFA salt) **17** (59.2 mg, 0.0643 mmol, 85 %) as a pink solid.

^{1}H NMR (400 MHz, CD_3OD): δ 8.40 (d, J = 8.2 Hz, 1H), 8.21 (dd, J = 1.8, 8.2 Hz, 1H), 7.88 (dd, J = 33.9, 10.5 Hz, 1H), 7.16 (d, J = 9.5 Hz, 2H), 7.06 (dd, J = 2.4, 9.5 Hz, 2H), 6.99 (d, J = 2.4 Hz, 2H), 3.75–3.70 (m, 2H), 3.69–3.55 (m, 20H), 3.31 (s, 12H), 3.15–3.11 (m, 2H). CO_2H, NH and NH_2 signals are missing.
MS (ESI) m/z: 693.2 $[M]^+$.

TAMRA-peg$_6$-BCN (TFA salt), 7

(*N*-(9-(5-((1-((1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-yl)-3-oxo-2,7,10,13,16,19-hexaoxa-4-azahenicosan-21-yl)carbamoyl)-2-carboxyphenyl)-6-(dimethylamino)-3*H*-xanthen-3-ylidene)-*N*-methylmethanaminium 2,2,2-trifluoroacetate)



$C_{50}H_{61}F_3N_4O_{13}$

MW = 983.03 g/mol

To a solution of TAMRA-peg$_6$-NH$_2$ **17** (TFA salt) (1 eq., 17 mg, 0.018 mmol) and TEA (5 eq., 0.013 mL, 0.092 mmol) in DMF (2 mL) was added (1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate **15** (1.1 eq., 6.4 mg, 0.020 mmol). The reaction was stirred at room temperature for 3 hours. After evaporation under reduced pressure, the crude material was purified by preparative HPLC to afford TAMRA-peg$_6$-BCN (TFA salt) **7** (12.2 mg, 0.012 mmol, 67 %) as a pink solid. ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.82 (t, J = 5.5 Hz, 1H), 8.29 (d, J = 8.2 Hz, 1H), 8.24 (dd, J = 1.7 and 8.2 Hz, 1H), 7.88 (d, J = 1.3 Hz, 1H), 7.12–7.00 (m, 5H), 6.97 (s, 2H), 4.01 (d, J = 8.0 Hz, 2H), 3.63–3.33 (m, 22H), 3.26 (s, 12H), 3.09 (q, J = 6.0 Hz, 2H), 2.30–2.01 (m, 6H), 1.64–1.40 (m, J = 9.6 Hz, 2H), 1.33–1.13 (m, 1H), 0.83 (t, J = 9.6 Hz, 2H). CO_2H signal is missing.
MS (ESI) m/z: 869.4 $[M]^+$.

2 Microfluidic experiments

2.1 Materials and methods

Microchip fabrication:
Silicon wafer fabrications were performed by STnano Platform (Hicham Majjad, IPCMS Strasbourg University). A mold of SU-8 resist (MicroChem Corp.) was prepared on a silicon wafer (Siltronix) by UV exposure (MJB3 contact mask aligner; SUSS MicroTec) through a photolithography mask (Selba SA) and subsequent development (SU-8 developer; MicroChem Corp.). A curing agent was added to the PDMS base (Sylgard 184 silicone elastomer kit; Dow Corning Corporation) to a final concentration of 10% (w/w), mixed and poured over the mold to a depth of 5 mm. Following degassing for several minutes and cross-linking at 70 °C for 2 h, the PDMS was peeled off the mold and the input and output ports were punched with a 0.75 mm-diameter Harris Uni-Core biopsy punch (Electron Microscopy Sciences). The PDMS was activated by incubation for 3 minutes in an oxygen plasma (Diener Zepto) and was bound to a 50 mm x 75 mm glass slide (Fisher Bioblock). Channels were made fluorophilic using a commercial surface coating agent (ABCR, AB111155). Height of the channel was 40 µm and size of the nozzle was 25 µm with a channel width of 40 µm. A second chip was used to reinject collected emulsion with a channel width of 50 µm.

Microfluidic station:
If not mentioned, all optical materials were purchased from Thorlab. The optical setup comprises an Eclipse Ti inverted microscope (Nikon) mounted on an optical table and includes 4 lasers (Strasus-375 nm 16mW, Stradus-488 nm 50 mW, Stradus-532 nm 40 mW and Stradus-642 nm 110 mW). Emitted fluorescence was detected by photomultiplier tubes (PMT, Hamamatsu Photosensor H10722-20). The output signal from the PMTs was analyzed using a PCI-7852R Virtex-5 LX50R FPGA card (National Instruments Corporation) executing a program written in LabView 2013 (FPGA module, National Instruments Corporation). The optical table includes also a camera (Guppy F-080, Allied Vision Technologies).

Confocal microscopy:
W/o emulsions were analyzed using a Leica SPE confocal microscope (lasers used: 405 nm (ACMS), 561 nm (TAMRA derivatives) and 635 nm (sulfoCy5 derivatives), objective 20X, Leica 11506513).

Fluorescence polarization:
W/o emulsions (15–30 µL) were put on Corning® 96 Well Half Area Microplates 3686. Fluorescence polarization analyses were performed in triplicates using Wallac® Victor 3 Multilabel Reader (Excitation/emission wavelengths 620/665 nm).

2.2 Experimental setup:

Flow rates were controlled by syringe pumps (Harvard Apparatus PHD 2000).
Flow rates of 500 µL/h for aqueous phase and of 500 µL/h for fluorinated oil phase (3M HFE 7500) were used to create droplets of 40-50 pL. Emulsion was collected in an Eppendorf filled with oil and closed with a PDMS plug to prevent coalescence due to contact with air. For control and azide surfactant dilution experiments, 2.5% w/w of non-functionalized surfactant (008-FluoroSurfactant, RAN Biotechnologies) was used in oil phase. For SPAAC reaction, the azide diblock surfactant Krytox-peg$_{12}$-azide **1** was used at 2.5% w/w in oil phase. TAMRA-peg$_6$-BCN **7**, sulfoCy5-BCN **6** and control fluorophores (TAMRA-6-CO_2H and Cy5-alkyne) were introduced *via* the aqueous phase and dissolved in Pluronic F-127 (0.01% in PBS 1x).
W/o emulsions were reinjected in the second chip and spaced by fluorous oil (3M HFE 7500). Flow rates of 200 µL/h for HFE 7500 and of 100 µL/h for emulsion sample were used.
ACMS used as fluorophore control was synthesized according to the literature.[7]

2.3 Additional data

Emulsion collected for fluorescence polarization experiments were reinjected in a second chip and analysed by confocal microscopy to validate click reaction at the inner surface.

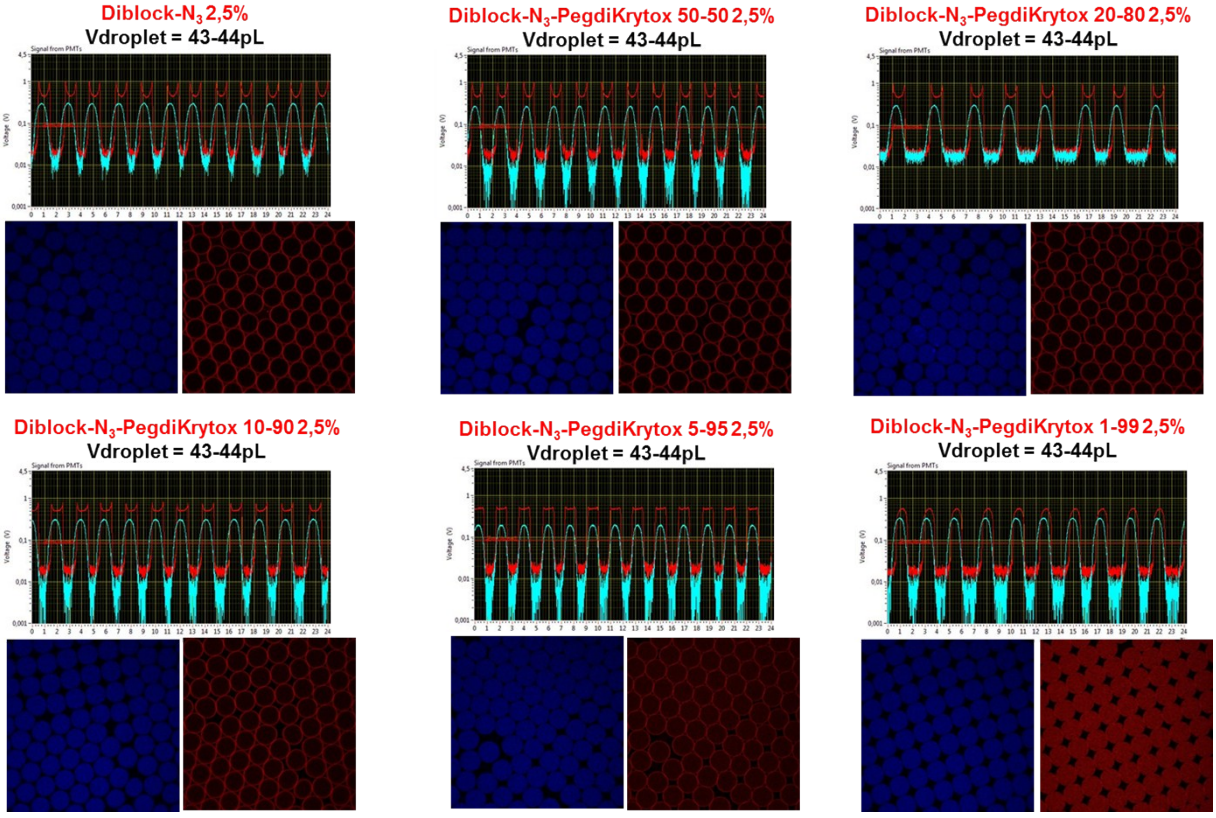


Figure S4: Control experiments for fluorescence polarization analysis. Signals obtained from PMT during emulsion reinjection, Red: laser 642 nm, Blue: laser 375 nm. Confocal microscopy, Red: laser 635 nm, Blue: laser 405 nm, Image size: 367.83 µm × 367.83 µm.

[7] G. Woronoff, A. El Harrak, E. Mayot, O. Schicke, O. J. Miller, P. Soumillion, A. D. Griffiths and M. Ryckelynck *Anal. Chem*., 2011, **83**, 2852.