Supporting information

Contents

List of abbreviations:

ABDA: 9,10-anthracenediyl-bis(methylene)dimalonic acid; ACN: acetonitrile; CPG: controlled pore glass; DCC: *N,N'*-Dicyclohexylcarbodiimide; DIPEA: (*N,N*-diisopropylethylamine; DMF: dimethylformamide; DMSO: dimethyl sulfoxide; DMS(O)MT: dimethoxymethylsulfonyltrityl; DMT: dimethoxytrityl; Et_2O: diethyl ether; HBTU: 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; MALDI-TOF: matrix-assisted laser desorption/ionization; NHS: *N*-hydroxysuccinamide; ON: oligonucleotide; PAGE: polyacrylamide gel electrophoresis; PS: photosensitiser; RP HPLC: reverse phase high-performance liquid chromatography; TBE: Tris/Borate/EDTA; TCA: trichloroacetic acid; TEAA: triethylammonium acetate; TFA: trifluoroacetic acid;

1. Materials and methods

Reagents: All chemical reagents and solvents were purchased from Sigma Aldrich unless otherwise stated. DNA reagents and the 5'-DMS(O)MT-Amino-Modifier C6 were obtained from Glen Research. Ce6 was purchased from Livchem Logistics. Oligonucleotides were purchased from Eurogentec.

Chemical synthesis:

Furan phosphoramidite: The furan building block for incorporation into the modified oligonucleotide FON1 was synthesised as previously described.(1)

Photosensitisers synthesis: TriPyCOOH porphyrin (2) and TT1 phthalocyanine (3) were synthesised as previously described. Chlorin e6 (Ce6) was purchased from Livchem Logistics.

Oligonucleotide synthesis, purification and characterisation: Oligonucleotides were synthesized at 1 μmol scale on an ABI 394 DNA synthesizer.

Furan modified oligonucleotide: Synthesis (DMT-On) proceeded through an automated phosphoramidite coupling cycle and was interrupted for a manual incorporation of the furan modified phosphoramidites. This coupling involved an alternating application of a dry 0,05 M solution of the modified phosphoramidite in acetonitrile and a dry 0,1 M dicyanoimidazole solution in acetonitrile, over 30 min. Then, and a mixture of Cap A (0.5 mL) and Cap B (0.5 mL) was pushed over the column, after which it was again flushed with acetonitrile (1 mL) and automated synthesis was resumed.

Amino modified oligonucleotide: The DMS(O)MT-amino modifier was installed on the synthesiser as an extra nucleobase. After incorporation at the 5' end of the growing ON,

the amino group was deprotected on column by treatment with 5 mL TCA, 10 mL ACN, 5 mL 20% Et_2NH in ACN and 10 mL of ACN.

Oligonucleotide cleavage and deprotection: The synthesized oligonucleotides were cleaved of the solid support by treatment with 1 mL of aqueous NH_4OH while shaking overnight at a temperature of 55°C.

Oligonucleotide purification: Purification with concurrent DMT removal of the synthesized oligonucleotides was carried out using a Sep-Pak C18 cartridge obtained from Waters. After equilibrating the cartridge using ACN and a 5 mM solution of TEAA, the oligonucleotides were loaded and the following solutions were eluted: an aqueous solution of NH_4OH (2.5%), milliQ water, an aqueous solution of TFA (1.5%), milliQ water and finally 20% ACN in water to elute the desired oligonucleotide. The fractions were combined and analyzed by RP HPLC and MALDI-TOF.

Oligonucleotide analysis: Oligonucleotides were analysed by RP-HPLC, using an Agilent 1200 system. The mobile phases consisted of A/acetonitrile and B/0,1 M TEAA buffer containing 5% acetonitrile. Furan and amino modified oligonucleotides were analysed using a Waters XBridge 130 Å Oligonucleotide C18 column (2,5 μm, 4,6 mm X 50 mm) used at 50°C (linear method 0-20% ACN in 30 min).

Oligonucleotide mass analysis: Masses of the oligonucleotides were determined by MALDI-TOF analysis on an ABI Voyager DE-STR MALDI-TOF. The oligonucleotide samples were mixed with a matrix consisting of 3-hydroxypicolinic acid and ammonium citrate present in a 9:1 ratio, in a 1:1 sample:matrix ratio. After mixing with the matrix, the samples were desalted by treatment with DOWEX beads. The samples were spotted on a MALDI plate, together with a commercial oligonucleotide sequence with a known mass (5'-GCA TCT CGT CAG-3'), purchased from Eurogentec, for calibration of the measurement.

Oligonucleotide concentration determination: Concentrations of the ONs solutions were measured on the Trinean DropSense96 UV/VIS droplet reader, using 2 μL sample for each measurement.

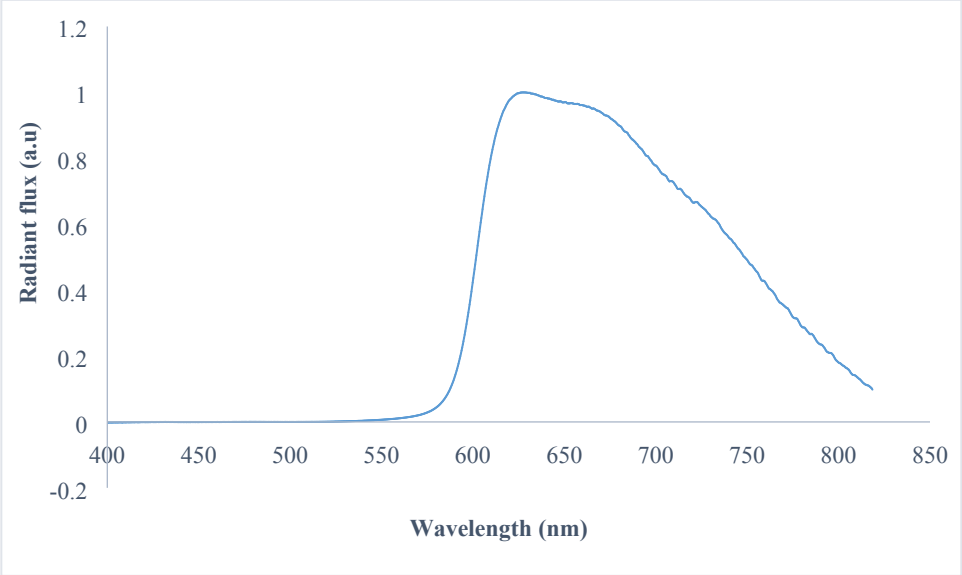
Synthesis of the PS conjugated ONs: Conjugation of the PS to the amino modified ONs was performed by activating the carboxylic acid functionality on the dyes and subsequent addition of the amino ON, as described in Section 4.

Analysis of the PS conjugated ONs: All conjugates were analysed and purified using RP HPLC equipped with a Discovery BIO Wide Pore C5 column (5 μm, 15 cm x 4,6 mm)

and the mobile phases consisted of A/acetonitrile and B/0,1 M TEAA buffer containing 5% acetonitrile.

Analysis of the PS conjugated ONs: Conjugates were characterised using MALDI-TOF MS (*vide supra*).

Crosslinking protocol: Crosslinking reactions where the photosensitisers were externally added were carried out at 20 µM oligonucleotide concentration and also at 2µM, for comparison with the crosslinking experiments where the photosensitiser conjugated oligonucleotides were used. The oligonucleotides were dissolved in a 10 mM phosphate buffer (pH 7) containing 100 mM of NaCl. The externally added photosensitisers or the photosensitiser conjugated oligonucleotides were added just before starting the irradiation. During the crosslinking reaction the temperature was kept constant at 25°C in an Eppendorf Thermomixer comfort with constant shaking at 950 rpm. Samples were irradiated using an Euromex fiber optic 100 W halogen light source Ek-1 equipped with a red filter and placed 1 cm above the sample. The spectrum when the red filter is placed was determined:



Crosslinking analysis: Samples were taken at different times for RP HPLC and PAGE analysis. After the maximum crosslinking yield was obtained, prolonged irradiation resulted in degradation of the crosslinked species. The chromatograms depicted here represent the maximum yield obtained in each experiment.

RP-HPLC analysis: Crosslinking samples where the photosensitiser was externally added were analysed by RP-HPLC equipped with a XBridge C18 column. Crosslinking samples with the photosensitizer conjugated to the oligonucleotide were measured with the RP-

HPLC equipped with a Discovery Wide Pore C5 column. Crosslinking yields were determined by integration of the corresponding peaks in the HPLC chromatogram and by comparing the area of the peak of the crosslinked species with the peak of the limiting single oligonucleotide strand, both corrected for their extinction coefficient. The extinction coefficient of the duplex was calculated based on the method described by R. Owczarzy *et al.*(4).

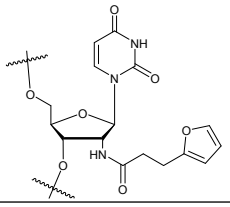
Gel electrophoresis: Samples were analysed by denaturing gel electrophoresis. The gels were prepared by dissolving 4,2 g urea in 5 mL of 40% acrylamide:bisacrylamide (19:1) and 1 mL 10x TBE buffer. 100 µL of a 0,5 M solution of ammonium persulphate was added and the solution was diluted to 10 mL with milliQ water. After cooling the solution, N,N,N',N'- tetramethylethylenediamine was added and the resulting mixture was poured between glass plates and allowed to polymerize for one hour. The gels were subjected to a pre-run in the consort EV202 at a voltage power of 225V during half an hour. After mixing the samples with formamide in 9:1 formamide:sample ratio (or 3:1 ratio for the experiments at 2 µM concentration of the oligonucleotide), they were loaded on the gel. During a run of approximately one hour, the temperature was kept at 25°C with a Julabo F12. The gels were stained with GelRed™ or GelGreen™ Nucleic acid gel stain (VWR) and photographed using an Autochemi imaging system.

Thermal Denaturation Experiments: All UV experiments were recorded on a Varian Cary 300 Bio instrument equipped with a six-cell thermostatted cell holder. Melting curves were monitored at 260 nm with a heating rate of 0.3 ºC/min. The buffer contained 100 mM NaCl and 10 mM phosphate buffer (pH 7). Oligonucleotide concentration was 1 µM for each strand. Melting temperatures were calculated from the first derivative of a tenth order polynomial function that fitted the data.

Singlet oxygen determination: 2µM of the PS conjugated ONs were dissolved in a 10 mM phosphate buffer (pH 7) containing 100 mM of NaCl. ABDA was added before irradiation (100µM) and samples were taken at 0, 0.5, 1, 2, 3, 4 and 5 min. Samples were measured using a Trinean DropSense96 UV/VIS droplet reader, using 2 µL samples for each measurement (each sampling was performed in duplicate).

2. Oligonucleotide characterisation

2.1 Furan modified oligonucleotide (FON1)

Name	Sequence (5'-3')	Modification
FON1	CTG ACG G**X**G TGC	X= 

a) b)

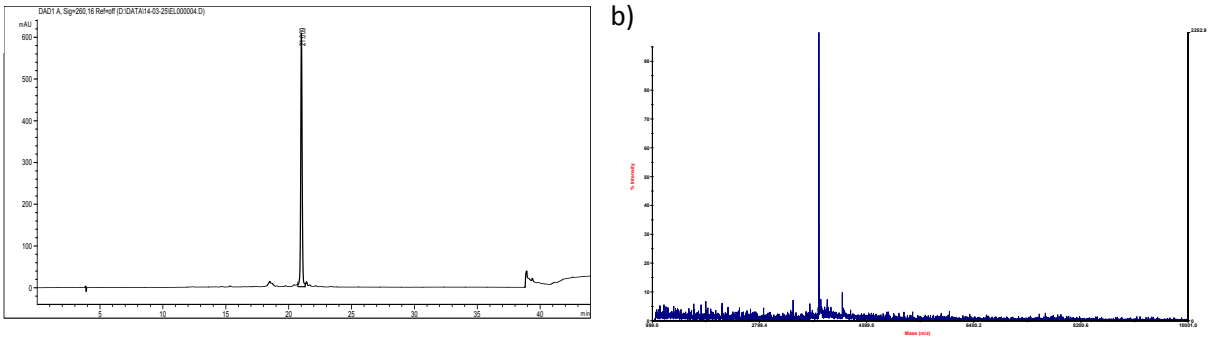


Figure S1. a) HPLC chromatogram of FON1. b) MALDI-TOF MS analysis. Expected mass: 3799 Da. Observed mass: 3799 Da

2.2 Complementary sequence (ON1)

As complementary sequence, the oligonucleotide 5'- GCA CCC CGT CAG - 3' was purchased from Eurogentec. For conjugation of the oligonucleotide to photosensitisers, an amino modified analogue of ON1 (NH_2-ON1) was purchased from Eurogentec or synthesized and kept on the CPG for further conjugation with one of the photosensitisers.

2.2.1 Amino modified oligonucleotide kept on solid support.

5'- $NH_2(CH_2)_6$- GCA CCC CGT CAG 3'

3 mg of the resulting CPG was deprotected for characterization whilst the rest was used for further functionalization with a photosensitiser.

a) b)

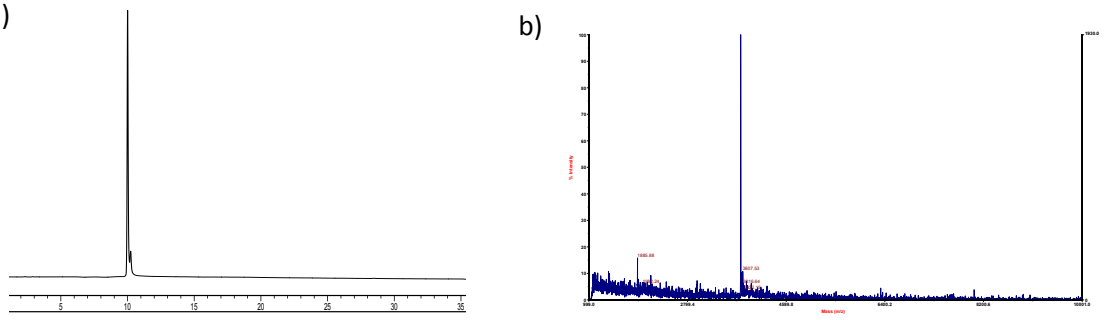


Figure S2. a) HPLC chromatogram of NH_2-ON1. b) MALDI-TOF MS analysis. Expected mass: 3769 Da. Observed mass: 3770 Da

3. **Crosslinking experiments using externally added photosensitisers**.

Stock solutions of the photosensitiser were prepared in DMSO (1,96 mM for TriPyCOOH; 923,76 μM for TT1; 2,18 mM for Ce6), diluted 10x in water (DMSO for TT1) and added to the solution immediately before irradiation.

3.1 Ce6

FON1 and ON1 were mixed together to a final concentration of 2 or 20 μM and Ce6 was added in DMSO to obtain a final PS concentration of 2, 5 or 10 μM.

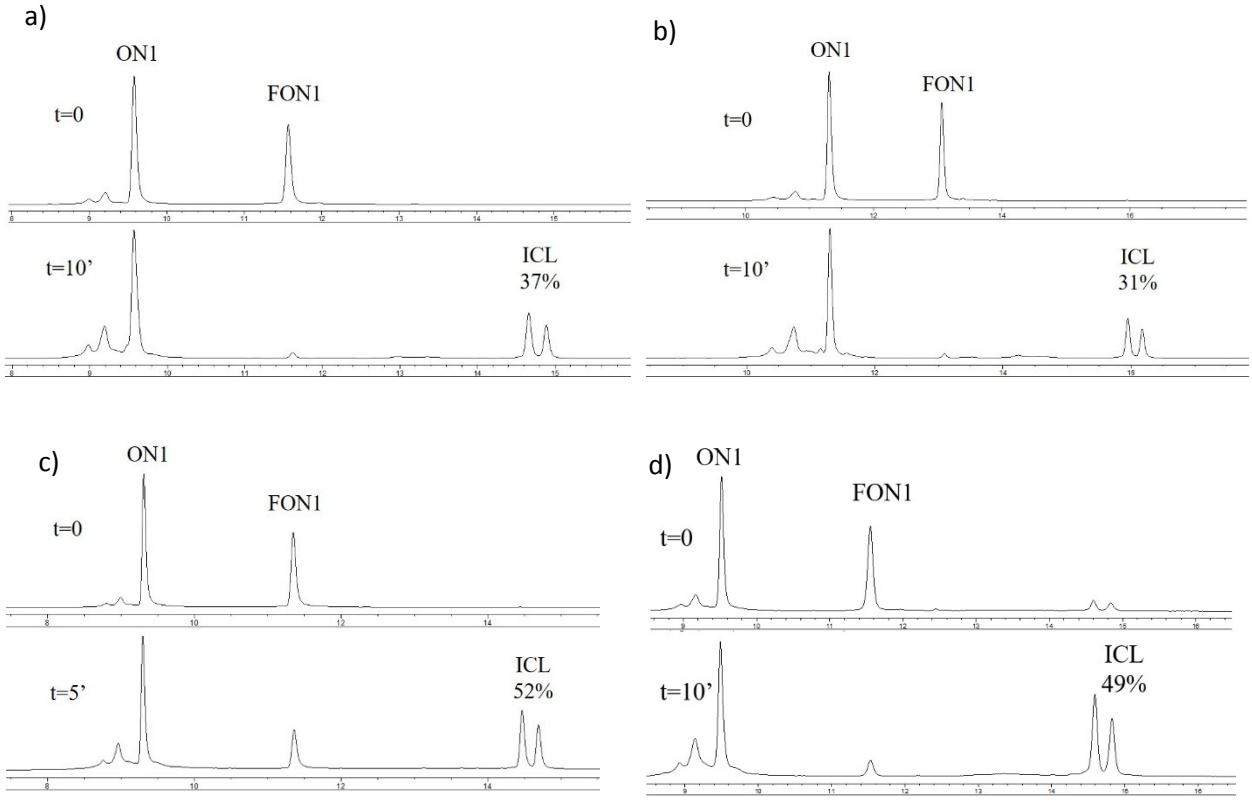


Figure S3. HPLC analysis of ICL experiments a) [ON] = 20 μM ON, [Ce6] = 5 μM. b) [ON] = 20 μM ON, [Ce6] = 10 μM. c) [ON] = 2 μM ON, [Ce6] = 2 μM; d) [ON] = 2 μM ON, [Ce6] = 2 μM 20% DMSO.

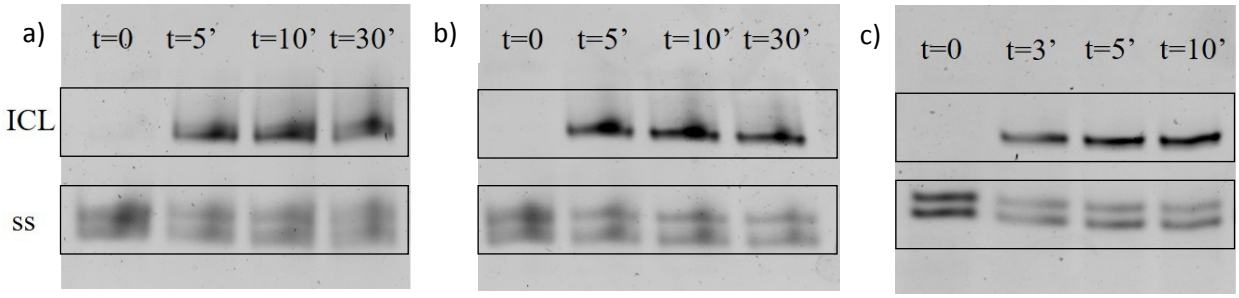


Figure S4. PAGE analysis (denaturing conditions) of ICL formation (ss: single strand ONs) a) [ON] = 20 μM ON, [Ce6] = 5 μM. b) [ON] = 20 μM ON, [Ce6] = 10 μM. c) [ON] = 2 μM ON, [Ce6] = 2 μM

3.2 TriPyCOOH

FON1 and ON1 were mixed together to a final concentration of 20 or 2 μM and TriPyCOOH was added in DMSO to obtain a final PS concentration of 2, 5 or 10 μM.

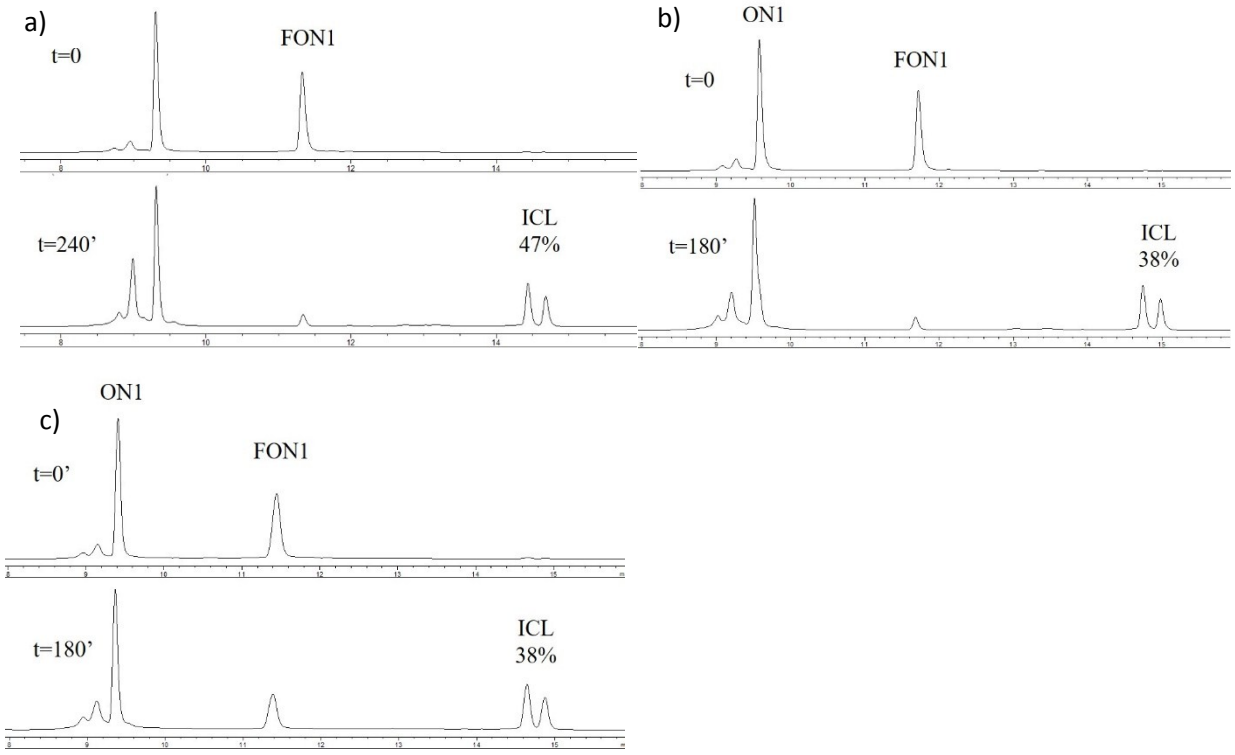


Figure S5. HPLC analysis of ICL experiments a) [ON] = 20 μM ON, [TriPyCOOH] = 5 μM. b) [ON] = 20 μM ON, [TriPyCOOH] = 10 μM. c) [ON] = 2 μM ON, [TriPyCOOH] = 2 μM.

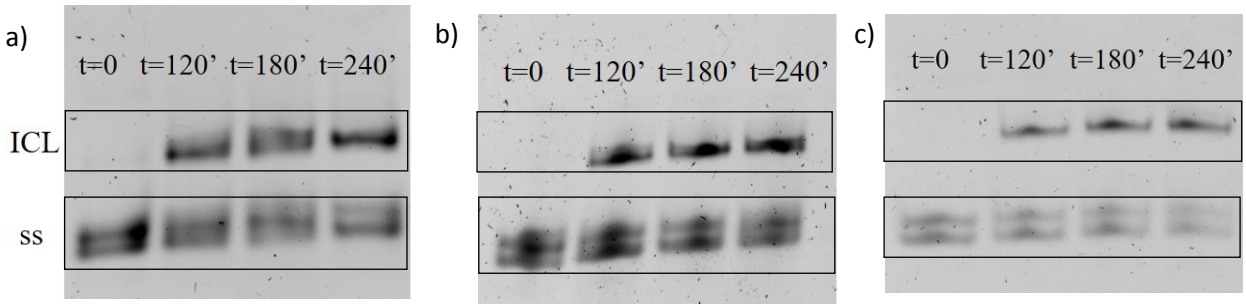


Figure S6. PAGE analysis (denaturing conditions) of ICL formation (ss: single strand ONs) a) [ON] = 20 μM ON, [TriPyCOOH] = 5 μM. b) [ON] = 20 μM ON, [TriPyCOOH] = 10 μM. c) [ON] = 2 μM ON, [TriPyCOOH] = 2 μM.

3.3 TT1

FON1 and ON1 were mixed together to a final concentration of 2 or 20 μM and TT1 was added in DMSO to obtain a final PS concentration of 2, 5, 10 or 20 μM.

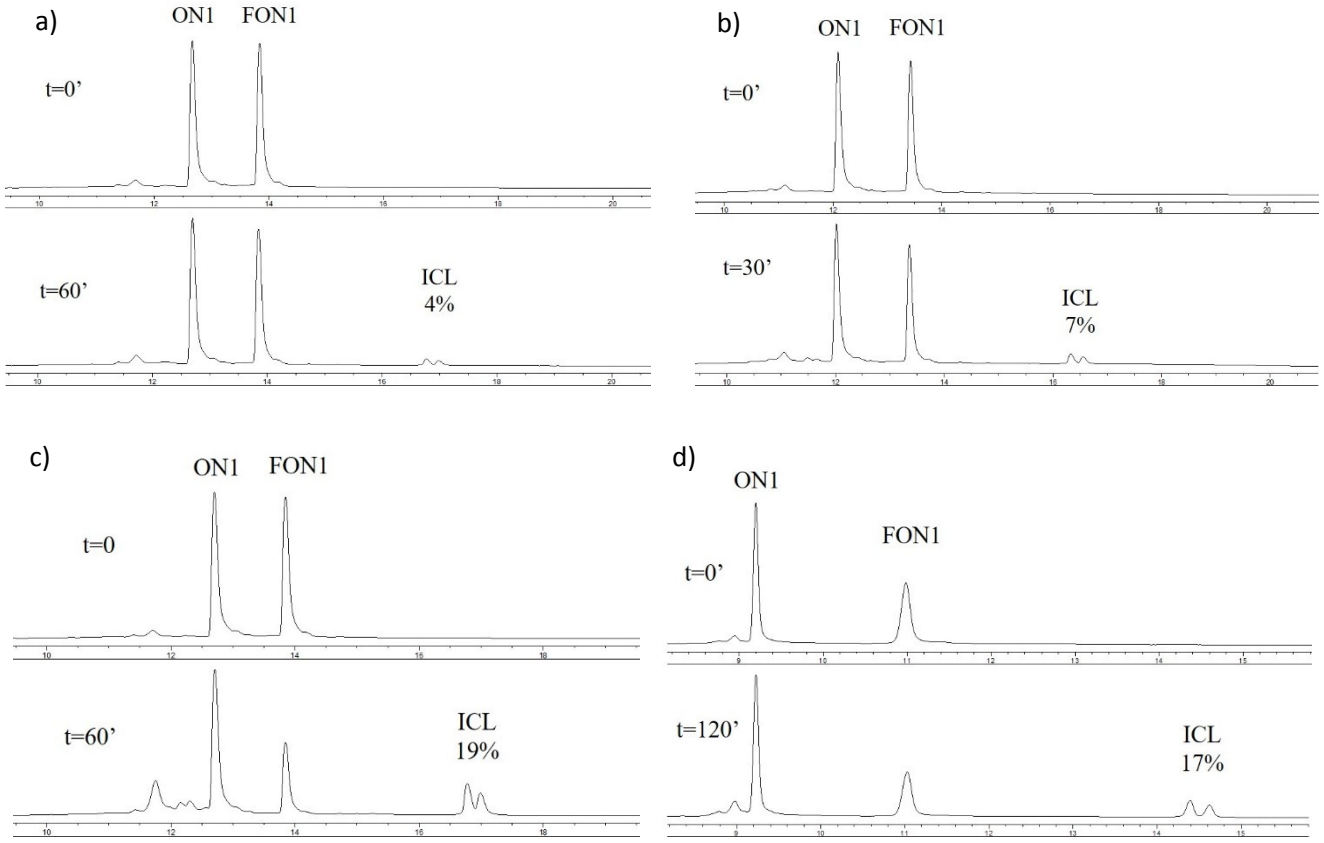


Figure S7. HPLC analysis of ICL experiments a) [ON] = 20 μM ON, [TT1] = 5 μM. b) [ON] = 20 μM ON, [TT1] = 10 μM. c) [ON] = 20 μM ON, [TT1] = 20 μM. d) [ON] = 2 μM ON, [TT1] = 2 μM.

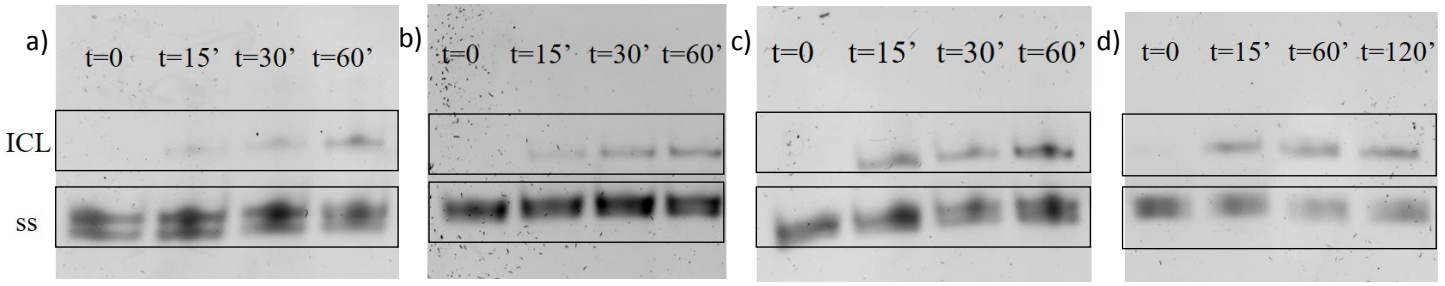
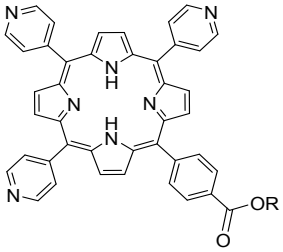
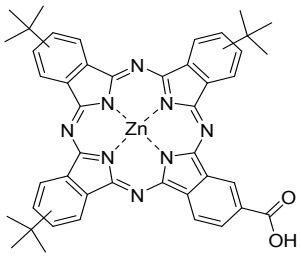


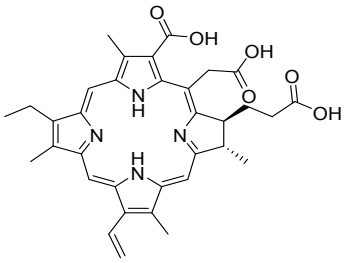
Figure S8. PAGE analysis (denaturing conditions) of ICL formation (ss: single strand ONs) a) [ON] = 20 μM ON, [TT1] = 5 μM. b) [ON] = 20 μM ON, [TT1] = 10 μM. c) [ON] = 20 μM ON, [TT1] = 20 μM. d) [ON] = 2 μM ON, [TT1] = 2 μM

4. Synthesis of the photosensitiser-conjugated oligonucleotides

Three different photosensitisers were evaluated during this work, either in solution or conjugated to an oligonucleotide. TriPyCOOH porphyrin (2) and TT1 phthalocyanine (3) were synthesised as previously described. Chlorin e6 (Ce6) was purchased from Livchem Logistics. The synthesis of the different conjugates is described below:







4.1 Chlorin e6 (PON1)

DCC (64 eq) and NHS (64 eq) were added in DMF to chlorin e6 (1 mg, 64 eq) and the solution stirred for 1 h in the darkness under Ar atmosphere. Then, an 0.1 M aqueous solution of $NaHCO_3$ (30 µL, pH 8.3) and the amino modified oligonucleotide (30 nmol) were added and the solution was stirred at room temperature for 18h in the darkness. Subsequently, 2x500 µL of mQ water were added and the resulting mixture centrifuged. The supernatant was collected, combined and lyophilised. The desired conjugate PON3 was obtained in a 30% yield by purification via RP-HPLC equipped with a Discovery BIO Wide Pore C5 column

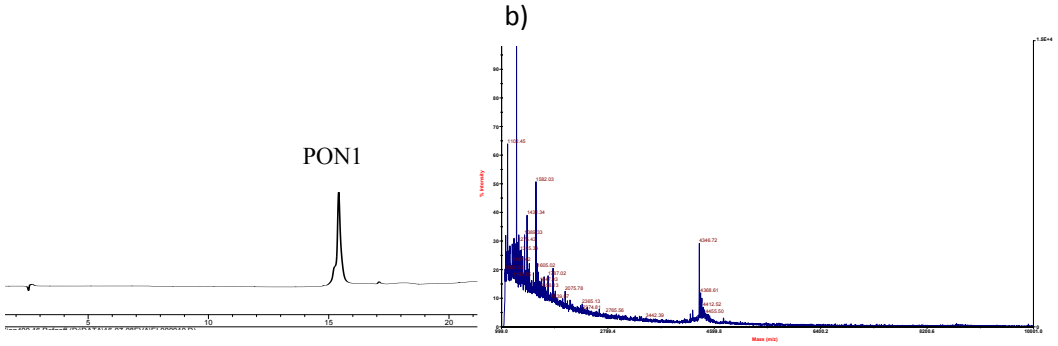


Figure S9. Figure. a) HPLC chromatograms of purified PON1 at 260 nm using a Discovery BIO Wide Pore C5 column. b) MALDI-TOF analysis. Observed mass: 4347 Da. Expected mass: 4347 Da

(linear gradient: 0-50% ACN in 15 min) and analysed by MALDI-TOF.

Table S1. PON1 absorption coefficients.

	PON1

Wavelength (nm)	410	645
ε (L·mol^{-1}·cm^{-1})	$1.66 \cdot 10^5$	$2.2 \cdot 10^4$

4.2 TriPyCOOH porphyrin (PON2)

TriPyCOOH was provided as the NHS ester (TriPyCOONHS). Conjugation with the amino modified oligonucleotide was carried out as follows: TriPyCOONHS (1 mg, 43 eq) was dissolved in DMSO (60 µL), then a 0.1 M aqueous solution of $NaHCO_3$ (30 µL, pH 8.3) and the amino modified oligonucleotide (30 nmol) were added and the solution stirred in the darkness at room temperature under Ar atmosphere for 18h. Subsequently, 2x500 µL of mQ water were added and the resulting mixture centrifuged. The supernatant was collected, combined and lyophilised. The desired conjugate PON2 was obtained in a 17% yield by purification via RP HPLC equipped with a Discovery BIO Wide Pore C5 or an Aeris Wide Pore colum (0-50% ACN in 15 min or 0-10% ACN in 15 min then 10-100% ACN in 15 min, repectively). PON2 was analysed by MALDI-TOF MS.

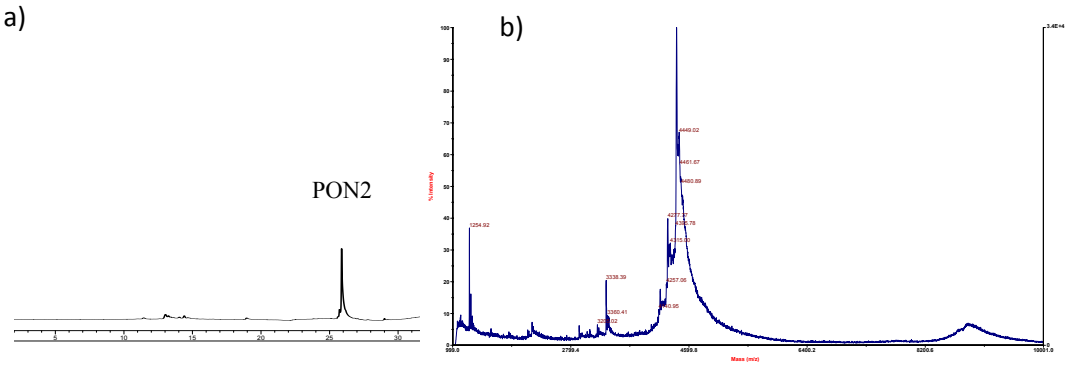


Figure S10. a) HPLC chromatograms of purified PON2 at 260 nm analysed using an Aeris Wide Pore column. b) MALDI-TOF analysis. Observed mass: 4412 Da. Expected mass: 4412 Da

Table S2. PON2 absorption coefficients.

	PON2				
Wavelength (nm)	425	520	555	590	650
ε (L·mol^{-1}·cm^{-1})	$1.82 \cdot 10^5$	$1.5 \cdot 10^4$	$7 \cdot 10^3$	$6 \cdot 10^3$	$3 \cdot 10^3$

4.3 TT1 phthalocyanine (PON3)

Conjugation using TT1 phthalocyanine was performed while the amino modified oligonucleotide was still attached to 1000 Å Control Pore Glass (CPG) as in the procedure followed by Kopecky *et al.*(5):

TT1 phthalocyanine (1 mg, 12 eq) was premixed with HBTU (10 eq) and DIPEA (6 µL) in DMF (30 µL) and added in 3 portions to the CPG (3 mg, aprox. 99 nmol of the

oligonucleotide), every 1h. The mixture was shaken for 24h at 55º in the darkness. Subsequently, the mixture was washed with 3x500 µL DMF, 3x500 µL ACN and 3x500 µL Et_2O, then dried. PON2 was then transferred to an Eppendorf and 1mL of NH_4OH was added and the mixture shaken overnight at 55 ºC in the darkness. The crude was pre-purified using Sep-Pak C18 cartridge (Waters) and the fractions eluted with 50% ACN were collected, combined and lyophilized.
The residue was purified via RP-HPLC equipped with a Discovery BIO Wide Pore C5 (linear gradient: 0-80% ACN in 15 min). PON2 was analysed by MALDI-TOF MS

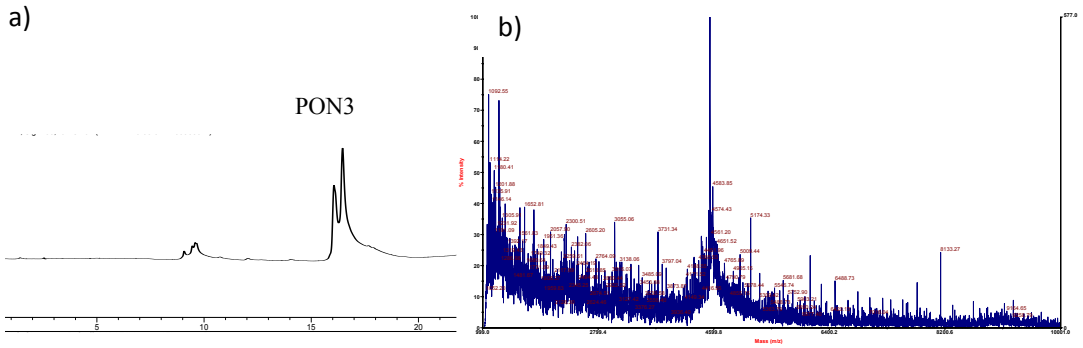


Figure S11. a) HPLC chromatograms of purified PON3 at 260nm analysed using a Discovery BIO Wide Pore C5 column. Double signal is appearing due to the fact that TT1 is used as a regioisomeric mixture. b) MALDI-TOF analysis. Observed mass: 4540 Da. Expected mass: 4539 Da.

Table S3. PON3 absorption coefficients

	PON3		
Wavelength (nm)	350	685	695
ε (L·mol^{-1}·cm^{-1})	$5.9 \cdot 10^4$	$1.17 \cdot 10^5$	$1.20 \cdot 10^5$

5. Melting Temperatures

5.1 Melting temperatures of the PS conjugated ONs

Table S4. Summary of melting temperatures of the PS conjugated ONs.

STRAND 1	STRAND 2	TM
FON1	ON1	35.8ºC
FON1	PON1	Tm=38.3ºC; Ta=34.2ºC
FON1	PON2	29.2ºC
FON1	PON3	33.5ºC

Ta= annealing temperature

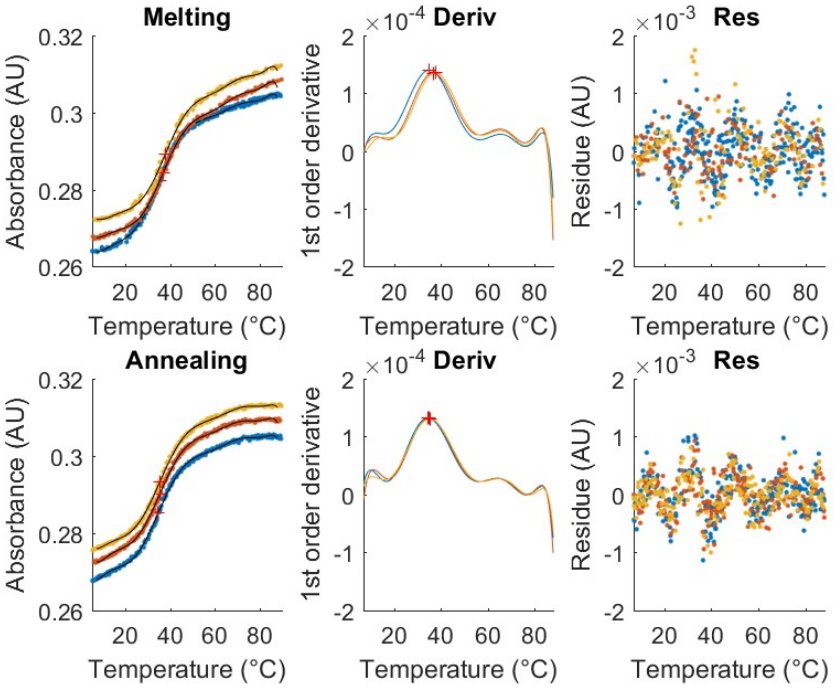


Figure S12. Melting temperatures of the FON1 and ON1 duplex.

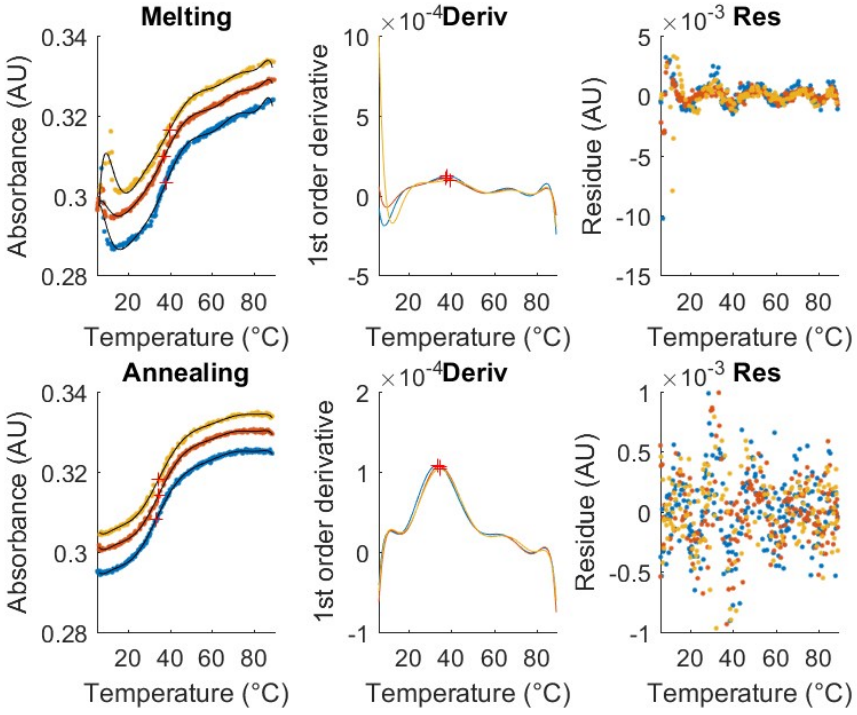


Figure S13. Melting temperatures of the FON1 and PON1 duplex.

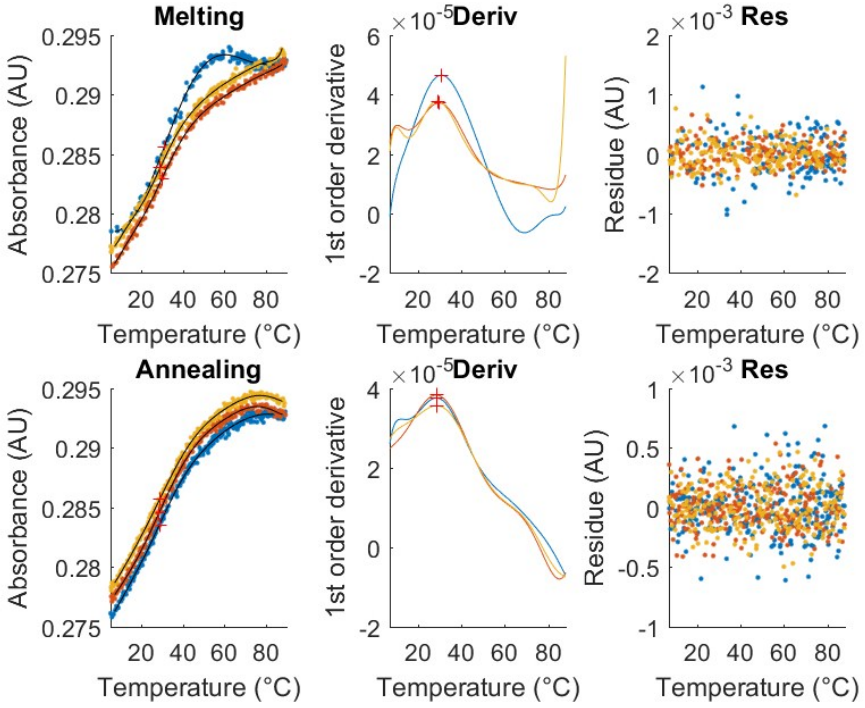


Figure S14. Melting temperatures of the FON1 and PON2 duplex.

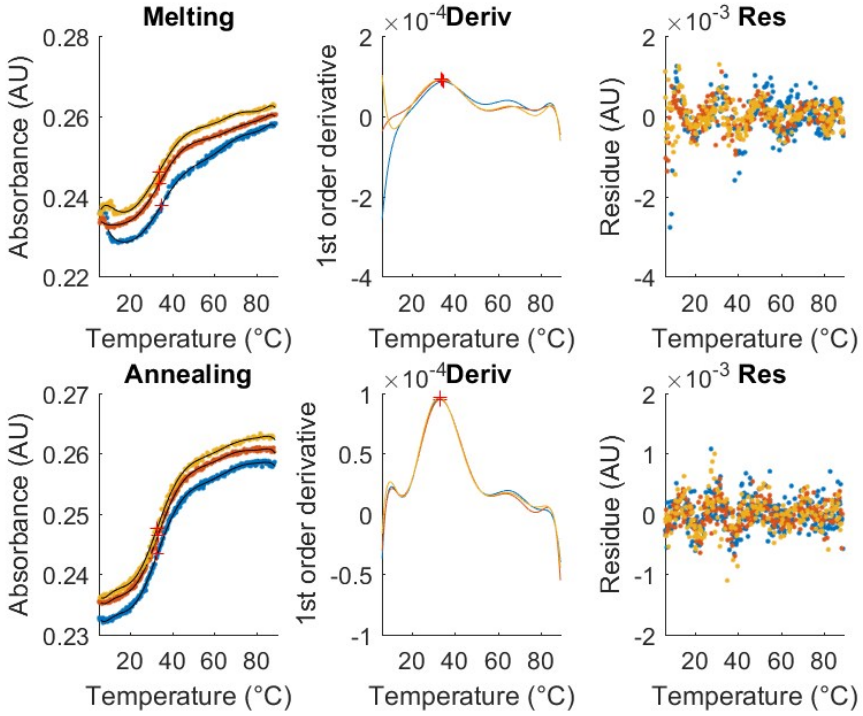


Figure S15. Melting temperatures of FON1 and PON3 duplex.

5.2 *Melting temperatures of unmodified duplex in the presence of DMSO*

Table S5. Melting temperatures of FON1-ON1 in the presence of different amounts of DMSO

DMSO %	TM
0	36.7
5	35.8
10	37.0
20	32.2

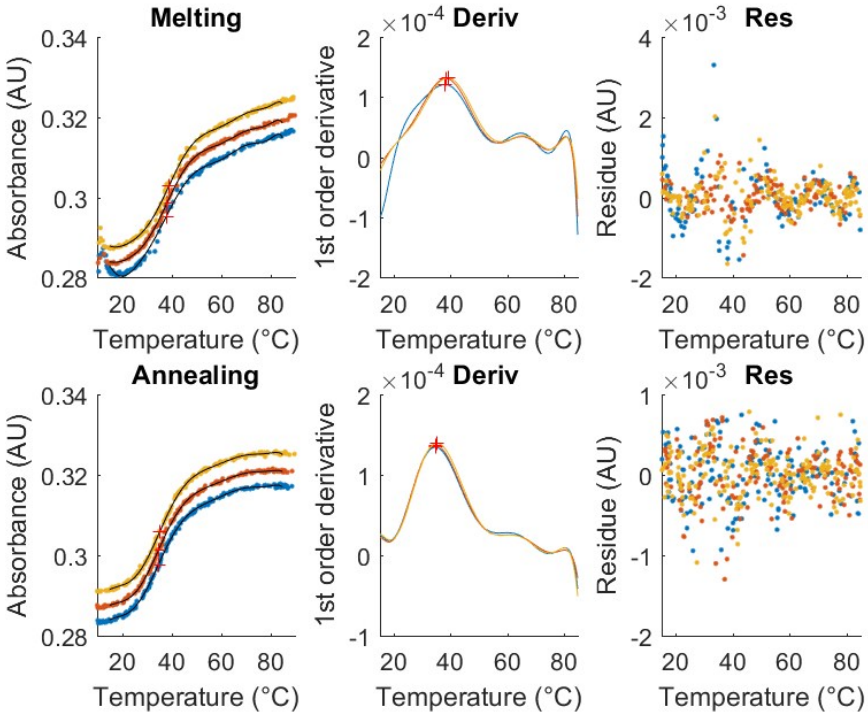


Figure S16. Melting temperatures of FON1-ON1 with no DMSO added.

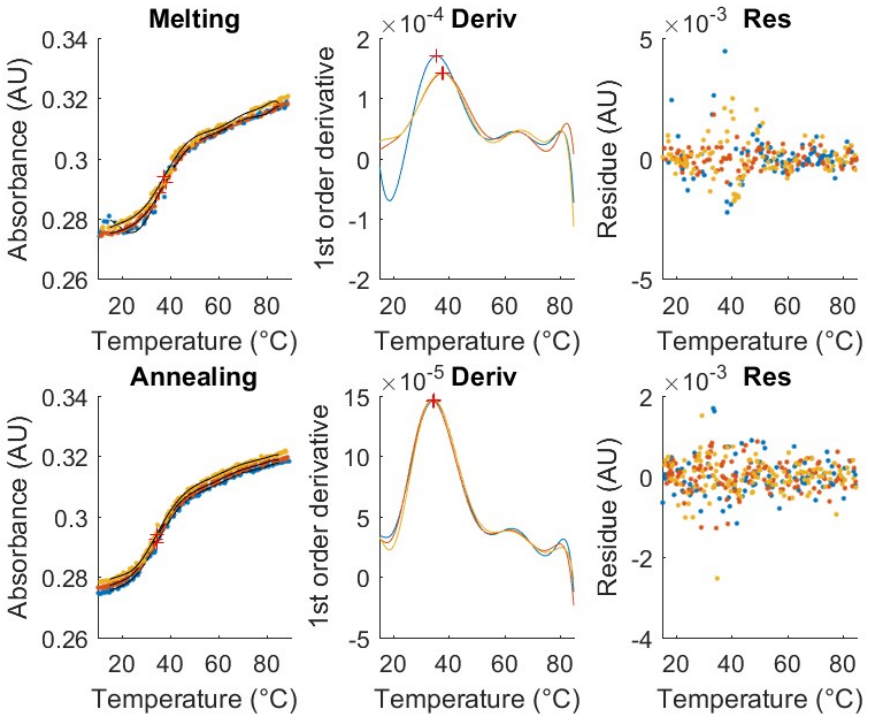


Figure S17. Melting temperatures of FON1-ON1 with 5% DMSO.

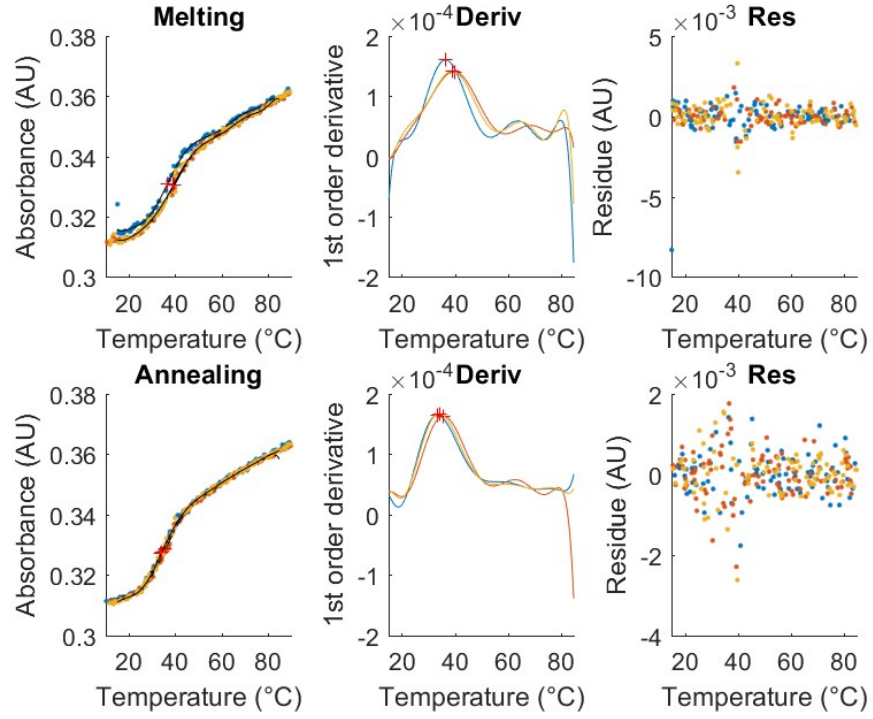


Figure S18. Melting temperatures of FON1-ON1 with 10% DMSO.

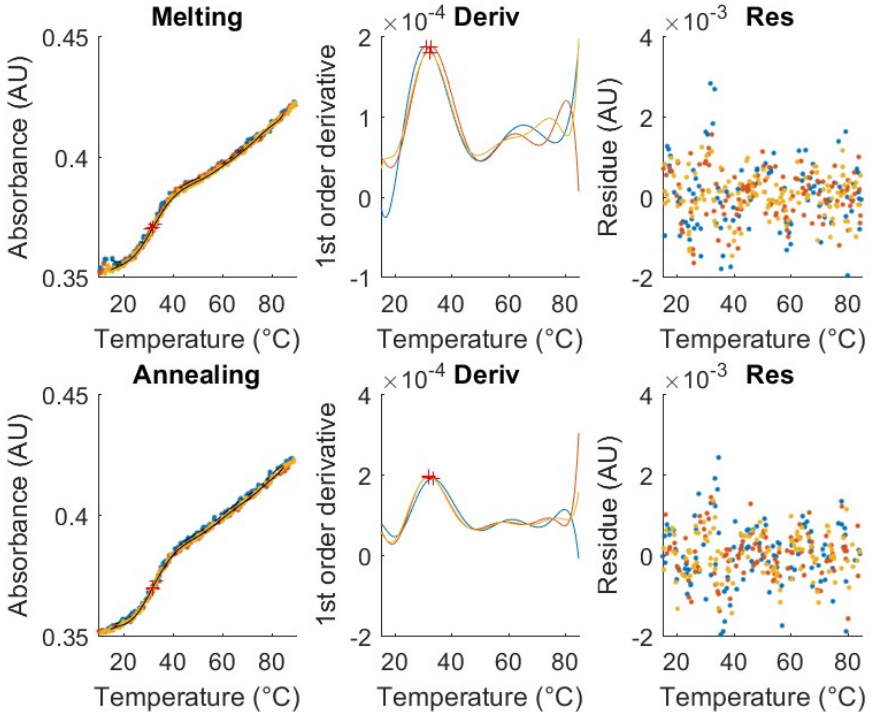


Figure S19. Melting temperatures of FON1-ON1 with 20% DMSO.

6. Crosslinking experiments with PS conjugated ON at lower temperature.

ICL formation using the conjugates was also evaluated at 10ºC. FON1 and the different PON conjugates were added at 2μM.

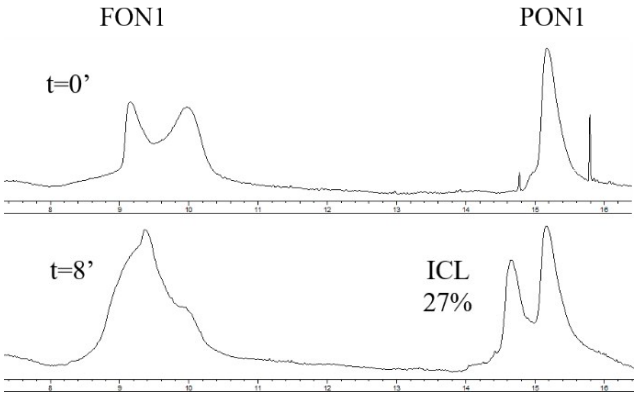


Figure S20. ICL experiment using PON1.

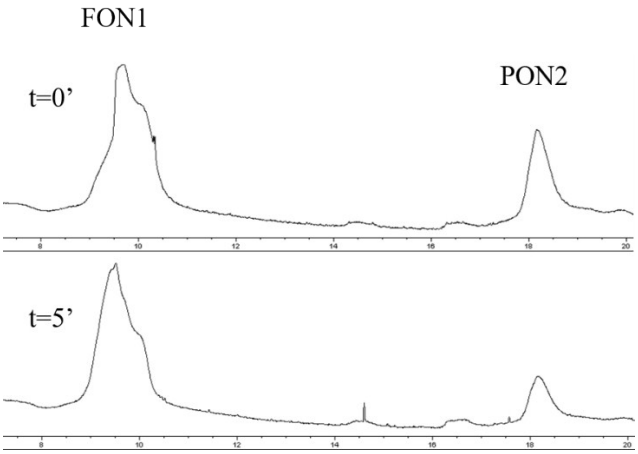


Figure S21. ICL experiment using PON2. No ICL was formed.

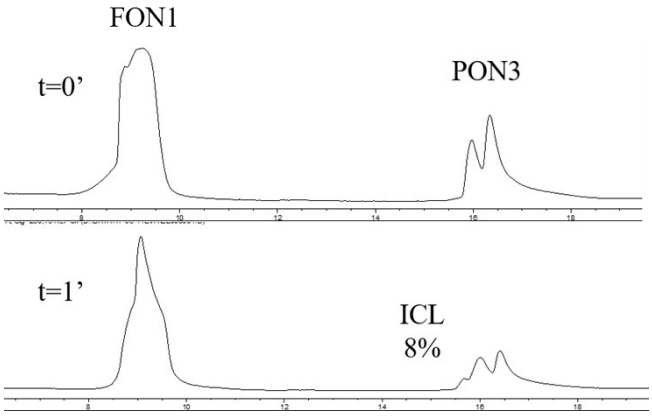


Figure S22. ICL experiment using PON3.

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