Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPORTING INFORMATION:

Dual fluorophore PNA FIT-Probes – extremely responsive and bright hybridization probes for the sensitive detection of DNA and RNA

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1. Synthesis

Loading of Novagen TGR Resin. The resin (250 mg, 0.29 µmol/g) was allowed to swell in 10 mL DMF for 30 min. For Fmoc removal the resin was twice treated with 1.5 mL of DMF/piperidine (4:1, v/v) and subsequently washed with DMF (5x2 mL), CH_2Cl_2 (5x2 mL) and DMF (5x2 mL). For preactivation PyBOP (130.1 mg, 250 µmol) and NMM (37.9 mg, 375 µmol) were added to a solution of Fmoc-protected glycine (74.3 mg, 250 µmol) in DMF (1.5 mL). After 3 min, the mixture was added to the resin. After 2.5 h, the resin was washed with DMF (5x2 mL), CH_2Cl_2 (5x2 mL) and DMF (5x2 mL). For capping, the resin was treated twice with 1.5 mL of a solution of Ac_2O/ pyridine (1:9, v/v). The resin was washed with DMF (5x2 mL) and CH_2Cl_2 (5x2 mL) and finally dried under reduced pressure.

Linear Solid Phase Synthesis. Fmoc-glycine loaded resin was allowed to swell in DMF. After 30 min, the resin was transferred to a synthesizer reactor. The resin was washed with DMF.

Fmoc Cleavage. A solution of DMF/piperidine (4:1) [v/v] was added to the resin. After 2 min the procedure was repeated. Finally the resin was washed with DMF.

Mmt Cleavage. The resin was first washed 10x with DCM and then treated with a solution of DCM/TRIS/TFA (93:5:2) [v:v:v] followed by washing 3x with DMF, 3x with pyridine, and 3x with DMF.

Capping. A solution of Ac_2O/ 2,6-lutidine/ DMF (5:6:89) [v/v/v] was added to the resin. After 2 min the resin was washed with DMF.

Coupling of Fmoc/Bhoc-protected PNA monomers. A preactivation vessel was charged with a 0.6 M HCTU solution in NMP, a 4 M NMM solution in DMF, and a 0.2 M PNA monomer solution in NMP. After 2 min, the preactivation solution was transferred to the resin. After 30 min, the resin was washed with DMF.

Coupling of Fmoc-Aeg(TO)-OH. A preactivation vessel was charged with a 0.6 M HCTU solution in NMP, a 4 M NMM solution in DMF, and a 0.2 M solution of **Fmoc-Aeg(TO)-OH**

and PPTS in NMP. After 2 min, the preactivation solution was transferred to the resin. After 30 min, the procedure was repeated and finally the resin was washed with DMF.

Coupling of amino acids. A preactivation vessel was charged with a 0.6 M HCTU solution in NMP, a 4 M NMM solution in DMF, and a 0.2 M amino acid monomer solution in NMP. After 2 min, the preactivation solution was transferred to the resin. After 30 min, the resin was washed with DMF.

Coupling of NIR667 succinimidylester. The resin was treated for 2x24h with a solution of 10 eq. NIR667 succinimidylester in DMF (final concentration 0.1 M) and 10 aq. NMP and subsequently washed three times with DMF, DCM, and DMF.

Coupling of NIR797 isothiocyanat. The resin was treated for 2x24h with a solution of 4 eq. NIR797 isothiocyanat in DMF (final concentration 0.1 M) and 4 aq. NMP and subsequently washed three times with DMF, DCM, and DMF.

Coupling of DY590 carboxylic acid. The resin was treated for 2x24h with a solution of 2 eq. DY590 carboxylic acid in DMF (final concentration 0.1 M), 1.8 eq. HCTU and 4 aq. NMP and subsequently washed three times with DMF, DCM, and DMF.

Coupling of DY752 carboxylic acid. The resin was treated for 2x24h with a solution of 2 eq. DY752 carboxylic acid in DMF (final concentration 0.1 M), 1.8 eq. HCTU and 4 aq. NMP and subsequently washed three times with DMF, DCM, and DMF.

Coupling of AlexaFluor594 carboxylic acid. The resin was treated for 1x12h with a solution of 2 eq. AlexaFluor594 carboxylic acid in DMF (final concentration 0.1 M), 1.8 eq. HCTU and 4 aq. NMP and subsequently washed three times with DMF, DCM, and DMF.

Coupling of ITCC carboxylic acid. The resin was treated for 2x12h with a solution of 2 eq. ITCC carboxylic acid in DMF (final concentration 0.1 M), 1.8 eq. HCTU and 4 aq. NMP and subsequently washed three times with DMF, DCM, and DMF.

Coupling of ICC carboxylic acid. The resin was treated for 2x12h with a solution of 2 eq. ICC carboxylic acid in DMF (final concentration 0.1 M), 1.8 eq. HCTU and 4 aq. NMP and subsequently washed three times with DMF, DCM, and DMF.

Cleavage. A solution of cysteine methyl ester hydrochloride (45 µmol) in 1.5 ml of a solution of TFA/*m*-cresol/H_2O (93:5:2) [v/v/v] was passed through the dried resin in 30 min. The resin was washed with TFA. The combined eluates were concentrated in vacuo.

Purification. To the concentrated cleavage solution was added cold diethyl ether. The precipitate was collected by centrifugation and disposal of the supernatant. The residue was dissolved in water and passed through a water-equilibrated Sep-pak® C18 cartridge. Colored fractions obtained upon elution with MeCN/ H_2O (70:30, v/v) were analyzed by HPLC and MALDI-TOF/MS and purified by semi preparative HPLC when the crude yield was > 5 nmol or by analytical HPLC when the crude yield was < 5 nmol. Gradient I: 3% B for 3 min, 3→20% B in 20 min. Gradient II: 3% B for 3 min, 3→30% B in 20 min. Gradient III: for 3 min, 3→50% B in 20 min.

The following fluorescence probes were synthesized in a 1 µM scale by using Fmoc-glycine loaded Novasyn TGR Rink-Amide resin (118 µmol/g).

H-Lys(H)-cgta Aeg(TO) atagccgatgccg-Gly-NH_2, 4

Yield: OD_{260} = 8.8 (46.8 nmol); $t_R^{II)}$ = 9.9 min;

MALDI-TOF-MS: m/z calcd. for $C_{214}H_{265}N_{108}O_{54}S_1$ $[M]^+$: 5288.2, found: 5286.2.

H-Lys(H)-gtccgta Aeg(TO) atagccgtcg-Gly-NH_2, 7

Yield: OD_{260} = 10.0 (53.6 nmol); $t_R^{II)}$ = 10.7 min;

MALDI-TOF-MS: m/z calcd. for $C_{214}H_{266}N_{105}O_{56}S_1$ $[M]^+$: 5279.1, found: 5277.2.

H-Lys(H)-gtcagccgta Aeg(TO) atagccg-Gly-NH_2, 10

Yield: OD_{260} = 7.6 (40.4 nmol); $t_R^{II)}$ = 11.7 min;

MALDI-TOF-MS: m/z calcd. for $C_{214}H_{265}N_{108}O_{54}S_1$ $[M]^+$: 5288.2, found: 5285.9.

H-Lys(H)-ccggtcagccgta Aeg(TO) atag-Gly-NH_2, 13

Yield: OD_{260} = 9.2 (49.6 nmol); $t_R^{II)}$ = 10.9 min;

MALDI-TOF-MS: m/z calcd. for $C_{214}H_{265}N_{108}O_{54}S_1$ $[M]^+$: 5288.2, found: 5285.1.

H-Lys(DY590)-cgta Aeg(TO) atagccgatgccg-Gly-NH_2, DY590-4

Yield: OD_{260} = 1.8 (9.6 nmol); $t_R^{II)}$ = 11.9 min;

MALDI-TOF-MS: m/z calcd. for $C_{251}H_{304}N_{111}O_{61}S_3$ $[M]^+$: 5947.0, found: 5947.1.

H-Lys(DY590)-gtccgta Aeg(TO) atagccgtcg-Gly-NH_2, DY590-7

Yield: OD_{260} = 2.0 (11.6 nmol); $t_R^{II)}$ = 11.5 min;

MALDI-TOF-MS: m/z calcd. for $C_{251}H_{305}N_{108}O_{63}S_3$ $[M]^+$: 5938.0, found: 5936.5.

H-Lys(DY590)-gtcagccgta Aeg(TO) atagccg-Gly-NH_2, DY590-10

Yield: OD_{260} = 2.6 (13.4 nmol); $t_R^{II)}$ = 12.3 min;

MALDI-TOF-MS: m/z calcd. for $C_{251}H_{304}N_{111}O_{61}S_3$ $[M]^+$: 5947.0, found: 5947.1.

H-Lys(DY590)-ccggtcagccgta Aeg(TO) atag-Gly-NH_2, DY590-13

Yield: OD_{260} = 1.8 (9.8 nmol); $t_R^{II)}$ = 12.5 min;

MALDI-TOF-MS: m/z calcd. for $C_{251}H_{304}N_{111}O_{61}S_3$ $[M]^+$: 5947.0, found: 5946.8.

H-Lys(Alex594)-cgta Aeg(TO) atagccgatgccg-Gly-NH_2, Alexa594-4

Yield: OD_{260} = 2.0 (10.8 nmol); $t_R^{II)}$ = 7.9 min;

MALDI-TOF-MS: m/z calcd. for $C_{208}H_{253}N_{106}O_{53}S_1$ $[M]^+$: 5949.9, found: 5952.4.

H-Lys(Alex594)-gtccgta Aeg(TO) atagccgtcg-Gly-NH_2, Alexa594-7

Yield: OD_{260} = 2.4 (12.4 nmol); $t_R^{II)}$ = 8.5 min;

MALDI-TOF-MS: m/z calcd. for $C_{208}H_{254}N_{103}O_{55}S_1$ $[M]^+$: 5940.9, found: 5940.1.

H-Lys(Alex594)-gtcagccgta Aeg(TO) atagccg-Gly-NH_2, Alexa594-10

Yield: OD_{260} = 2.6 (15.3 nmol); $t_R^{II)}$ = 8.0 min;

MALDI-TOF-MS: m/z calcd. for $C_{208}H_{253}N_{106}O_{53}S_1$ $[M]^+$: 5949.9, found: 5950.8.

H-Lys(Alex594)-ccggtcagccgta Aeg(TO) atag-Gly-NH_2, Alexa594-13

Yield: OD_{260} = 2.2 (11.4 nmol); $t_R^{II)}$ = 12.5 min;

MALDI-TOF-MS: m/z calcd. for $C_{208}H_{253}N_{106}O_{53}S_1$ $[M]^+$: 5949.9, found: 5951.9.

H-Lys(DY752)-cgta Aeg(TO) atagccgatgccg-Gly-NH_2, DY752-4

Yield: OD_{260} = 2.2 (11.4 nmol); $t_R^{III)}$ = 10.5 min;

MALDI-TOF-MS: m/z calcd. for $C_{257}H_{315}N_{110}O_{65}S_4$ $[M]^+$: 6112.2, found: 6112.0.

H-Lys(DY752)-gtccgta Aeg(TO) atagccgtcg-Gly-NH_2, DY752-7

Yield: OD_{260} = 2.0 (10.4 nmol); $t_R^{III)}$ = 11.2 min;

MALDI-TOF-MS: m/z calcd. for $C_{256}H_{316}N_{107}O_{67}S_4$ $[M]^+$: 6103.2, found: 6102.4.

H-Lys(DY752)-gtcagccgta Aeg(TO) atagccg-Gly-NH_2, DY752-10

Yield: OD_{260} = 2.6 (13.6 nmol); $t_R^{III)}$ = 10.7 min;

MALDI-TOF-MS: m/z calcd. for $C_{257}H_{315}N_{110}O_{65}S_4$ $[M]^+$: 6112.2, found: 6114.3.

H-Lys(DY752)-ccggtcagccgta Aeg(TO) atag-Gly-NH_2, DY752-13

Yield: OD_{260} = 3.0 (15.8 nmol); $t_R^{III)}$ = 16.4 min;

MALDI-TOF-MS: m/z calcd. for $C_{257}H_{315}N_{110}O_{65}S_4$ $[M]^+$: 6112.2, found: 6111.4.

H-Lys(NIR797)-ccggtcagccgta Aeg(TO) atag-Gly-NH_2, NIR797-13

Yield: OD_{260} = 0.5 (2.8 nmol); $t_R^{III)}$ = 14.7 min;

MALDI-TOF-MS: m/z calcd. for $C_{259}H_{319}N_{111}O_{59}S_5$ $[M]^+$: 6104.4, found: 6106.5.

The following fluorescence probes were synthesized in a 0.5 μM scale by using Fmoc-glycine loaded Novasyn TGR Rink-Amide resin (118 μmol/g).

H-Lys(NIR664)-cgta Aeg(TO) atagccgatgccg-Gly-NH_2, NIR664-4

Yield: OD_{260} = 3.4 (18.2 nmol); $t_R^{III)}$ = 13.5 min;

MALDI-TOF-MS: m/z calcd. for $C_{251}H_{305}N_{110}O_{58}S_2$ $[M]^+$: 5853.9, found: 5853.6.

H-Lys(NIR664)-gtccgta Aeg(TO) atagccgtcg-Gly-NH_2, NIR664-7

Yield: OD_{260} = 3.2 (17.0 nmol); $t_R^{III)}$ = 13.8 min;

MALDI-TOF-MS: m/z calcd. for $C_{251}H_{306}N_{107}O_{60}S_2$ $[M]^+$: 5844.9, found: 5842.4.

H-Lys(NIR664)-gtcagccgta Aeg(TO) atagccg-Gly-NH_2, NIR664-10

Yield: OD_{260} = 2.8 (15.1 nmol); $t_R^{III)}$ = 13.7 min;

MALDI-TOF-MS: m/z calcd. for $C_{251}H_{305}N_{110}O_{58}S_2$ $[M]^+$: 5853.9, found: 5852.9.

H-Lys(NIR664)-ccggtcagccgta Aeg(TO) atag-Gly-NH_2, NIR664-13

Yield: OD_{260} = 2.7 (14.3 nmol); $t_R^{III)}$ = 14.2 min;

MALDI-TOF-MS: m/z calcd. for $C_{251}H_{305}N_{110}O_{58}S_2$ $[M]^+$: 5853.9, found: 5853.4.

H-Lys(ICC)-cgta Aeg(TO) atagccgatgccg-Gly-NH_2, ICC-4

Yield: OD_{260} = 3.2 (17.0 nmol); $t_R^{II)}$ = 14.0 min;

MALDI-TOF-MS: m/z calcd. for $C_{246}H_{302}N_{110}O_{61}S_3$ $[M]^+$: 5870.9, found: 5872.2.

H-Lys(ICC)-gtccgta Aeg(TO) atagccgtcg-Gly-NH_2, ICC-7

Yield: OD_{260} = 3.4 (18.3 nmol); $t_R^{II)}$ = 14.5 min;

MALDI-TOF-MS: m/z calcd. for $C_{246}H_{303}N_{107}O_{63}S_3$ $[M]^+$: 5861.9, found: 5861.9.

H-Lys(ICC)-gtcagccgta Aeg(TO) atagccg-Gly-NH_2, ICC-10

Yield: OD_{260} = 3.6 (19.1 nmol); $t_R^{II)}$ = 15.2 min;

MALDI-TOF-MS: m/z calcd. for $C_{246}H_{302}N_{110}O_{61}S_3$ $[M]^+$: 5870.9, found: 5869.8.

H-Lys(ICC)-ccggtcagccgta Aeg(TO) atag-Gly-NH_2, ICC-13

Yield: OD_{260} = 3.9 (20.7 nmol); $t_R^{II)}$ = 14.2 min;

MALDI-TOF-MS: m/z calcd. for $C_{246}H_{302}N_{110}O_{61}S_3$ $[M]^+$: 5870.9, found: 5870.1.

H-Lys(ITCC)-cgta Aeg(TO) atagccgatgccg-Gly-NH_2, ITCC-4

Yield: OD_{260} = 4.3 (22.7 nmol); $t_R^{III)}$ = 11.8 min;

MALDI-TOF-MS: m/z calcd. for $C_{250}H_{306}N_{110}O_{61}S_3$ $[M]^+$: 5919.3, found: 5920.5.

H-Lys(ITCC)-gtccgta Aeg(TO) atagccgtcg-Gly-NH_2, ITCC-7

Yield: OD_{260} = 4.6 (24.5 nmol); $t_R^{III)}$ = 11.7 min;

MALDI-TOF-MS: m/z calcd. for $C_{250}H_{307}N_{107}O_{63}S_3$ $[M]^+$: 5910.3, found: 5910.9.

H-Lys(ITCC)-gtcagccgta Aeg(TO) atagccg-Gly-NH_2, ITCC-10

Yield: OD_{260} = 4.7 (25.1 nmol); $t_R^{III)}$ = 11.9 min;

MALDI-TOF-MS: m/z calcd. for $C_{250}H_{306}N_{110}O_{61}S_3$ $[M]^+$: 5919.3, found: 5921.4.

H-Lys(ITCC)-ccggtcagccgta Aeg(TO) atag-Gly-NH_2, ITCC-13

Yield: OD_{260} = 3.3 (17.6 nmol); $t_R^{III)}$ = 12.1 min;

MALDI-TOF-MS: m/z calcd. for $C_{250}H_{306}N_{110}O_{61}S_3$ $[M]^+$: 5919.3, found: 5924.6.

H-Lys(NIR797)-cgta Aeg(TO) atagccgatgccg-Gly-NH_2, NIR797-4

Yield: OD_{260} = 0.4 (2.3 nmol); $t_R^{III)}$ = 14.1 min;

MALDI-TOF-MS: m/z calcd. for $C_{259}H_{319}N_{111}O_{59}S_5$ $[M]^+$: 6104.4, found: 6107.2.

H-Lys(NIR797)-gtccgta Aeg(TO) atagccgtcg-Gly-NH_2, NIR797-7

Yield: OD_{260} = 0.7 (24.0 nmol); $t_R^{III)}$ = 14.6 min;

MALDI-TOF-MS: m/z calcd. for $C_{259}H_{320}N_{108}O_{61}S_5$ $[M]^+$: 6095.4, found: 6094.8.

H-Lys(NIR797)-gtcagccgta Aeg(TO) atagccg-Gly-NH_2, NIR797-10

Yield: OD_{260} = 0.6 (3.4 nmol); $t_R^{III)}$ = 14.6 min;

MALDI-TOF-MS: m/z calcd. for $C_{259}H_{319}N_{111}O_{59}S_5$ $[M]^+$: 6104.4, found: 6107.6.

The following fluorescence probes were synthesized in a 0.5 µM scale by using Fmoc-glycine loaded Novasyn TGR Rink-Amide resin (136 µmol/g).

H-Lys cagtta Aeg(TO) tatgccgttg-Lys-NH_2, NA10

Yield: OD_{260} = 3.0 (18.1 nmol); t_R[I)] = 9.9 min;

MALDI-TOF-MS: m/z calcd. for $C_{209}H_{264}N_{97}O_{55}S_1$ $[M]^+$: 5046.0, found: 5047.1.

H-Lys cagtta Aeg(TO) tatgccgttg-Lys(ICC)-NH_2, ICC-NA10

Yield: OD_{260} = 1.6 (9.1 nmol); t_R[I)] = 13.8 min;

MALDI-TOF-MS: m/z calcd. for $C_{241}H_{301}N_{99}O_{62}S_3$ $[M]^+$: 5671.8, found: 5672.1.

H-Lys cagtta Aeg(TO) tatgccgttg-Lys(ITCC)-NH_2, ITCC-NA10

Yield: OD_{260} = 0.8 (4.7 nmol); t_R[III)] = 11.0 min;

MALDI-TOF-MS: m/z calcd. for $C_{245}H_{305}N_{99}O_{62}S_3$ $[M]^+$: 5723.9, found: 5723.9.

2. HPLC analysis and MALDI-TOF-MS data

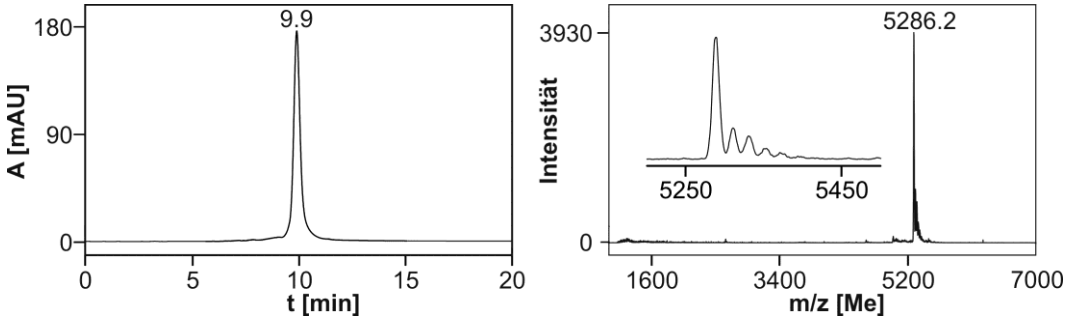


Figure S1 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **4**.

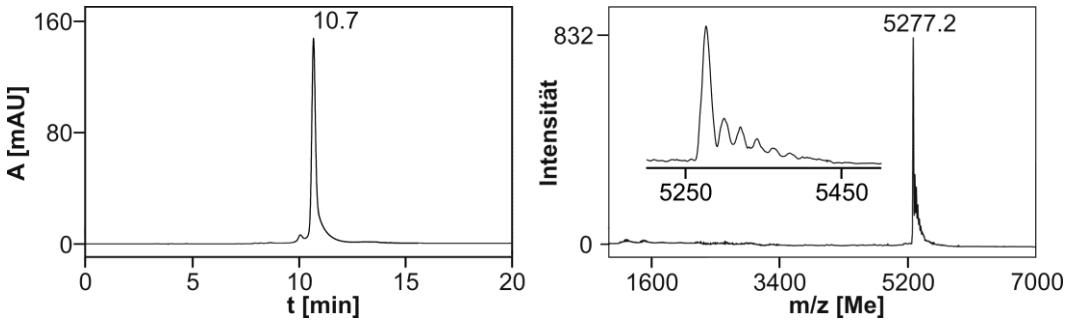


Figure S2 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **7**.

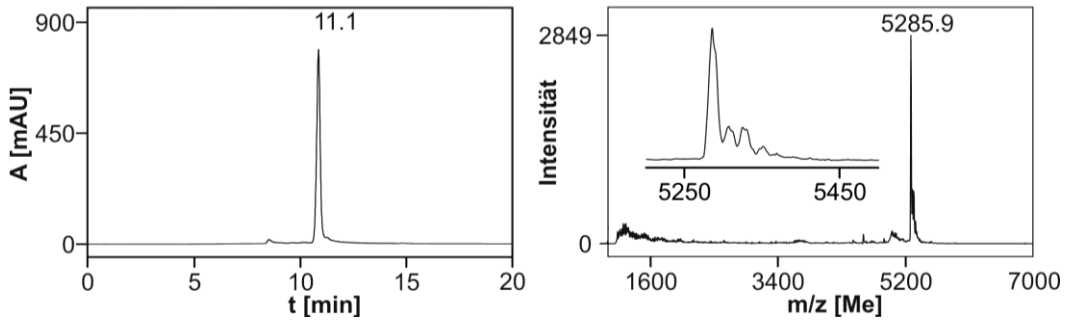


Figure S3 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **10**

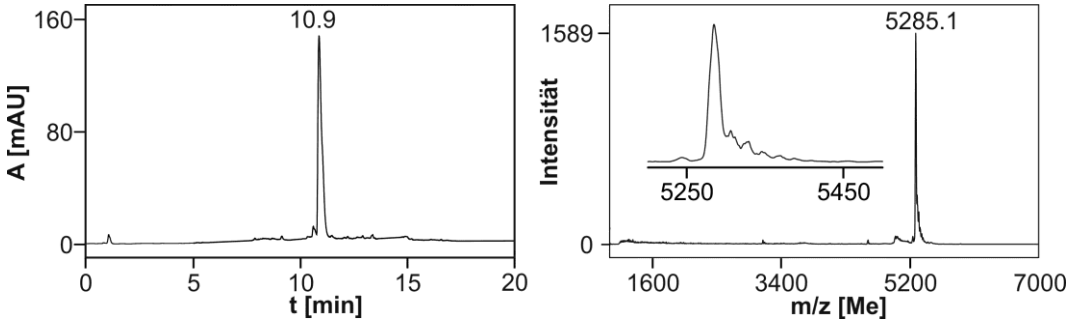


Figure S4 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **13**.

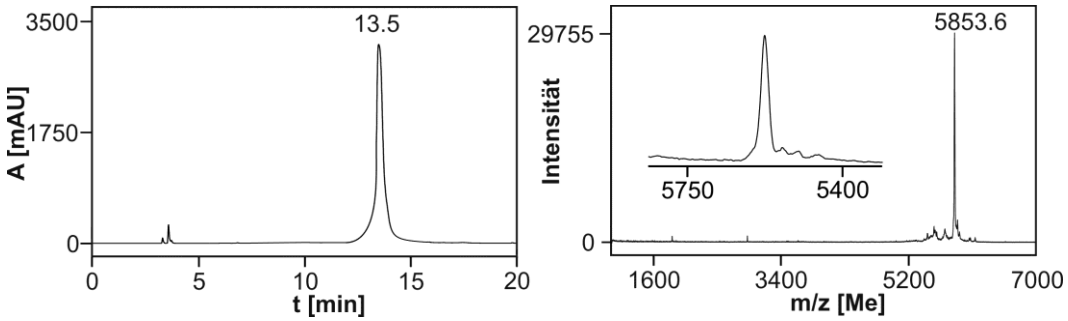


Figure S5 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **NIR664-4**.

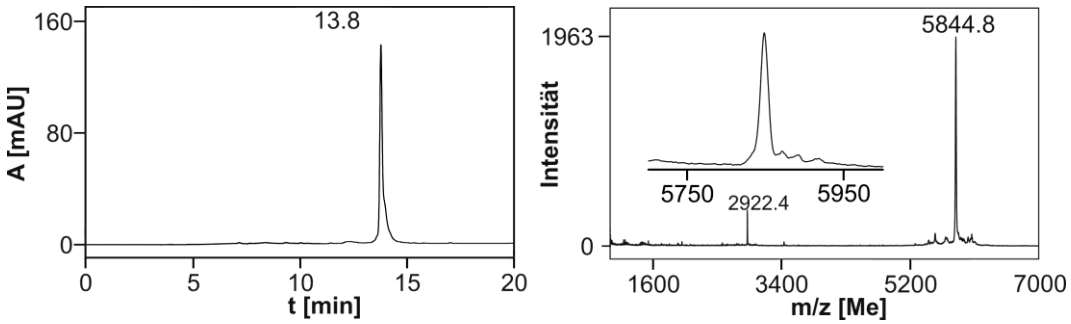
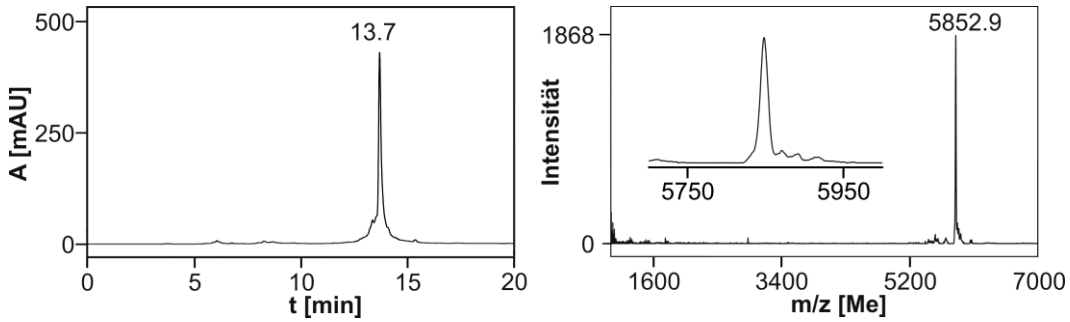


Figure S6 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **NIR664-7**



FigureS7 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **NIR664-10**.

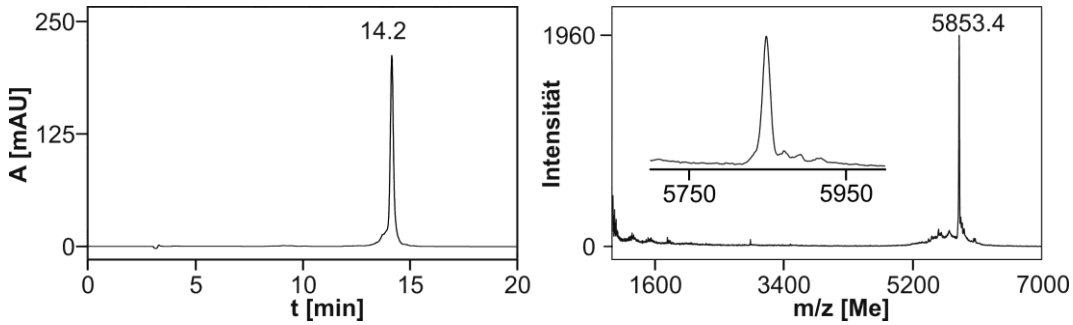


Figure S8 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **NIR664-13**.

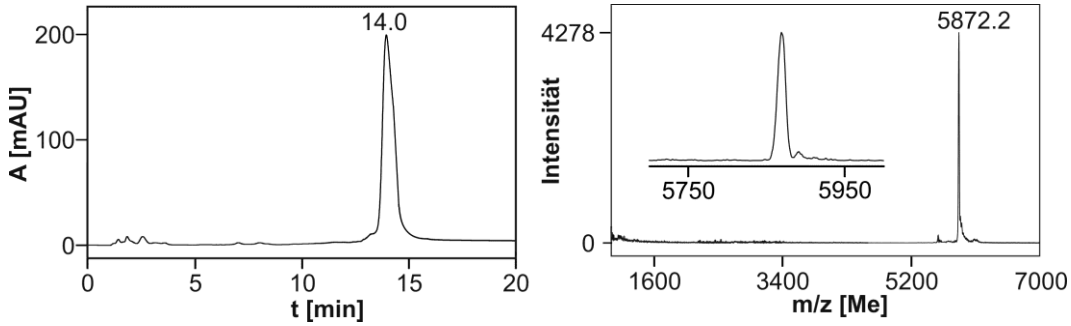


Figure S9 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **ICC-4**.

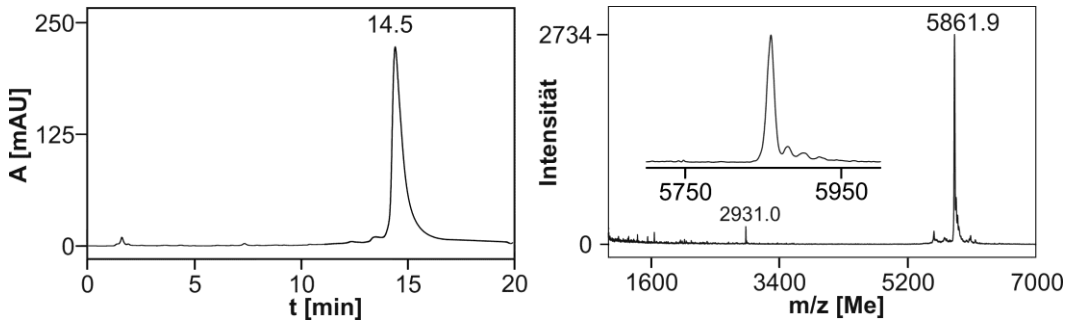


Figure S10 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **ICC-7**.

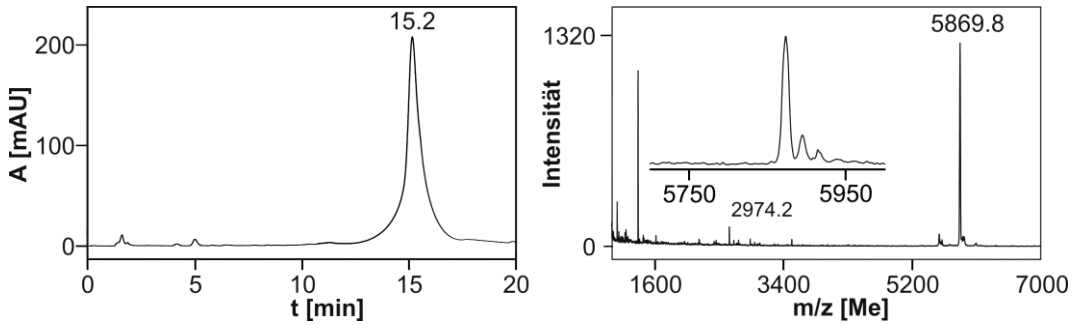


Figure S11 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **ICC-10**.

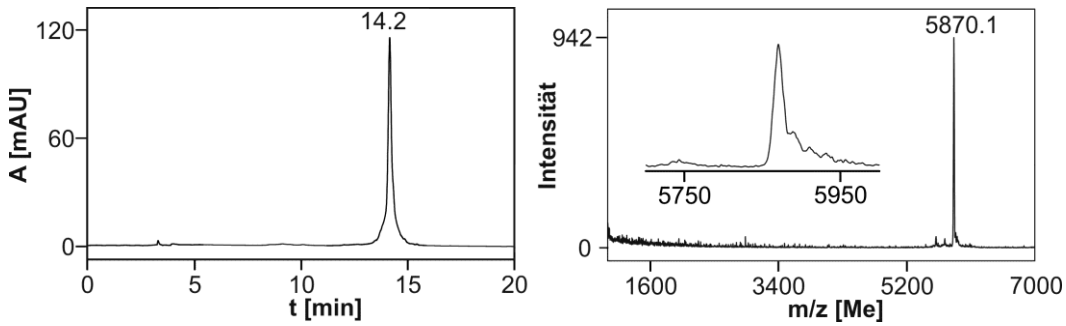


Figure S12 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **ICC-13**.

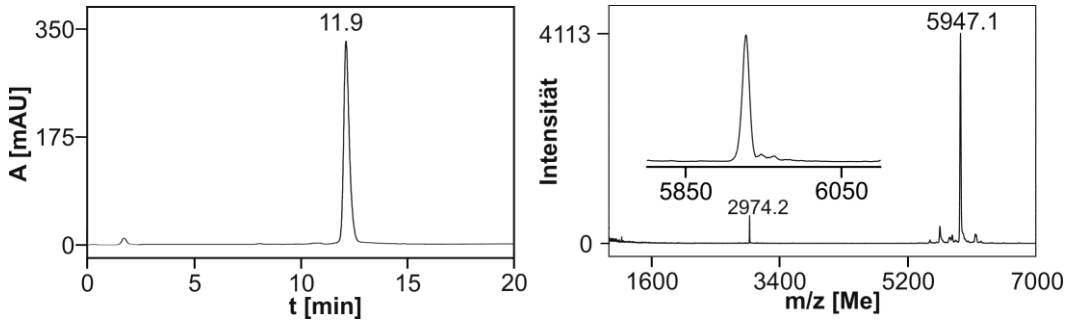


Figure S13 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **DY590-4**.

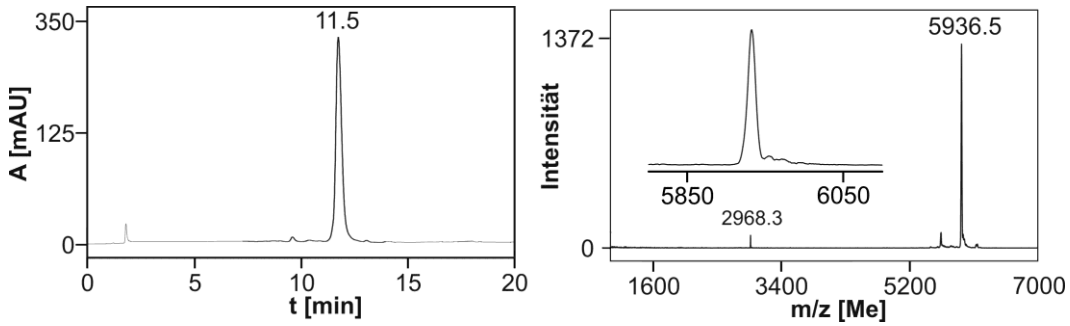


Figure S14 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **DY590-7**.

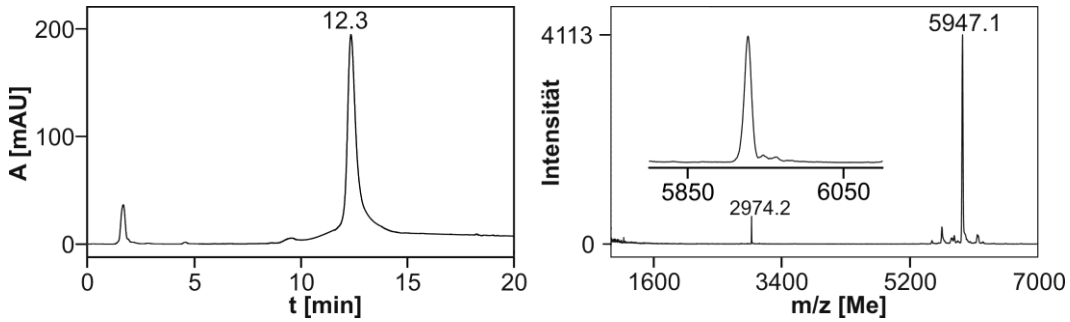


Figure S15 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **DY590-10**.

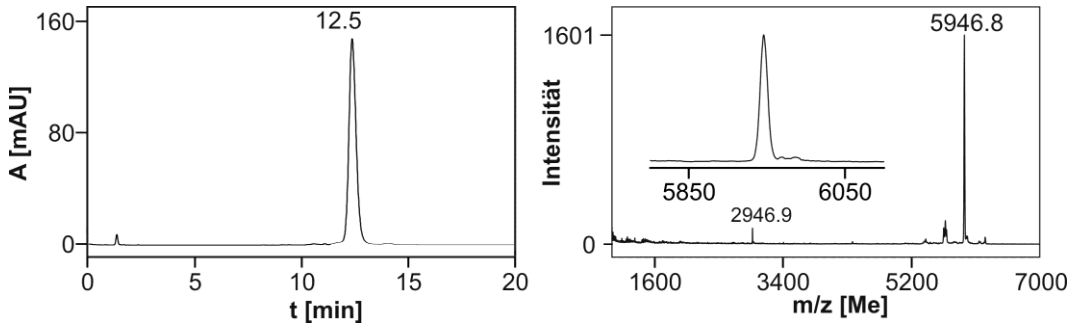


Figure S16 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **DY590-13**.

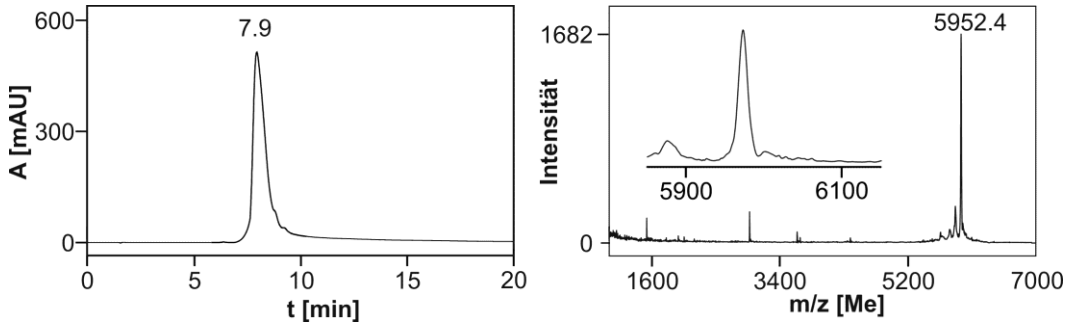


Figure S17 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **Alexa594-4**.

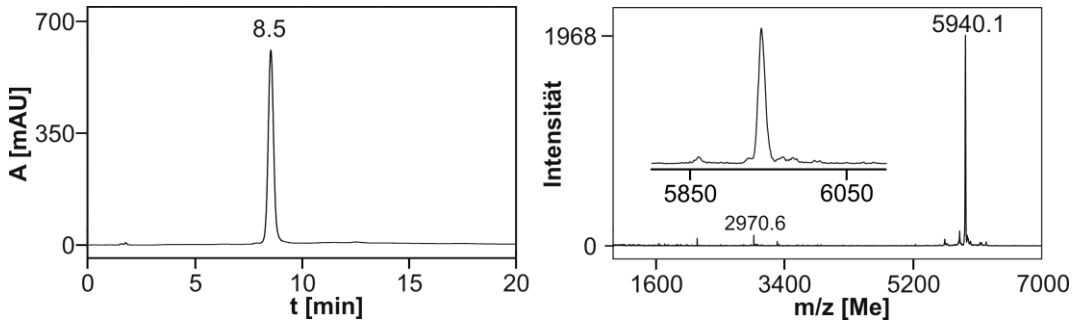


Figure S18 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **Alexa594-7**.

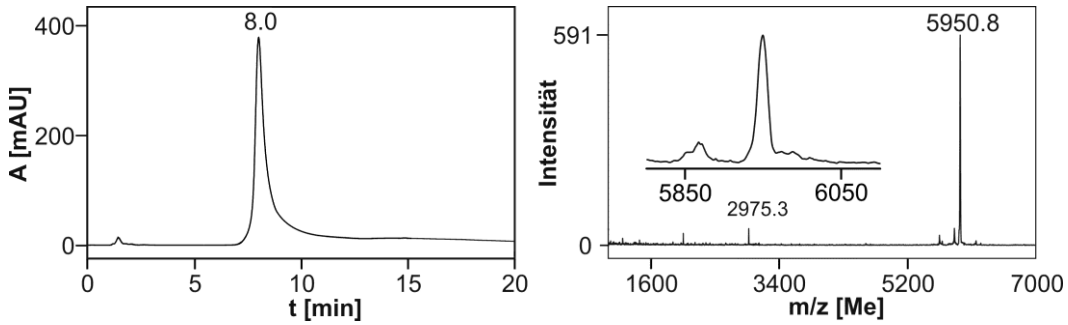


Figure S19 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **Alexa594-10**.

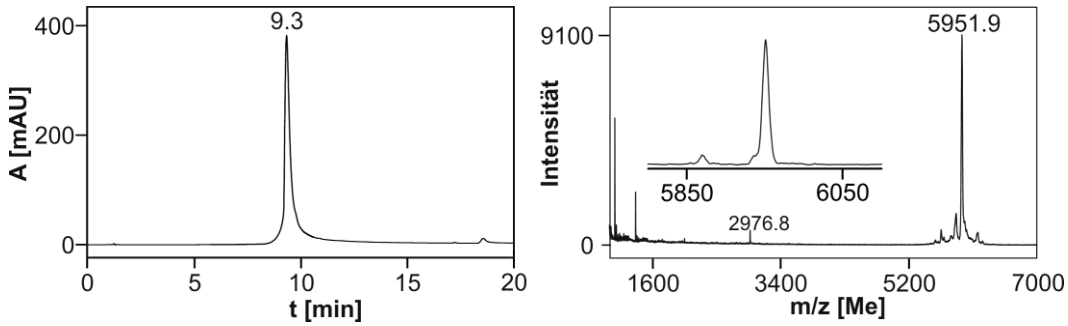


Figure S20 HPLC trace (Gradient II) and MALDI-TOF mass spectrum of probe **Alexa594-13**.

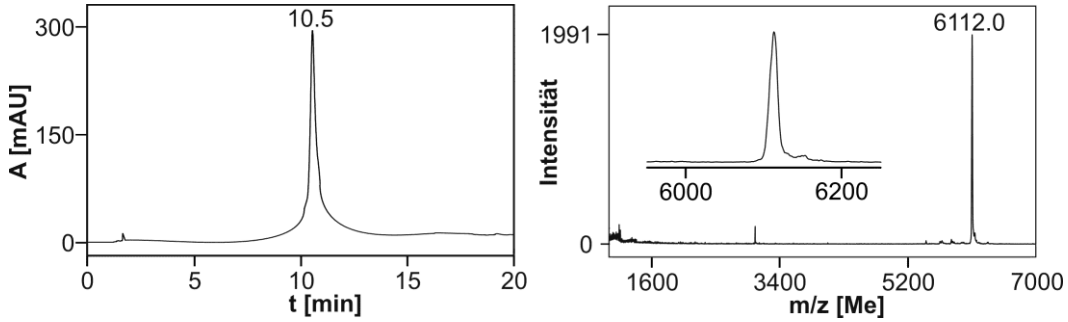


Figure S 21 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **DY752-4**.

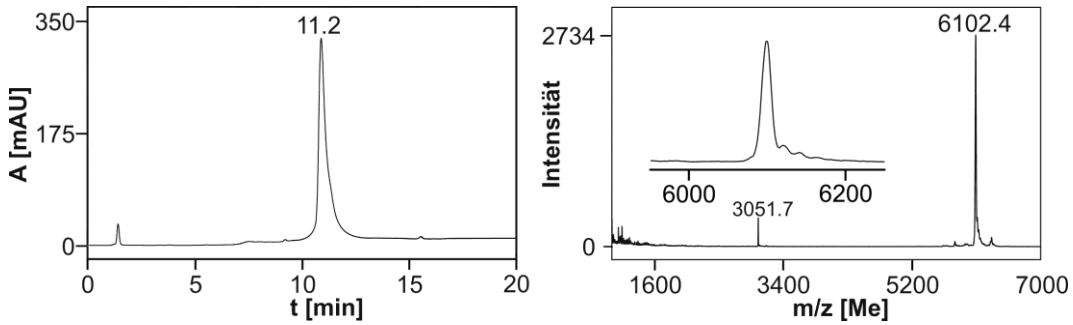


Figure S22 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **DY752-7**.

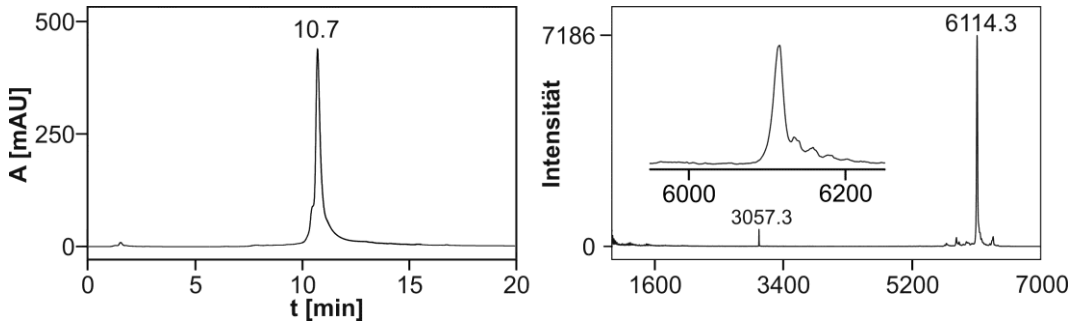


Figure S23 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **DY752-10**.

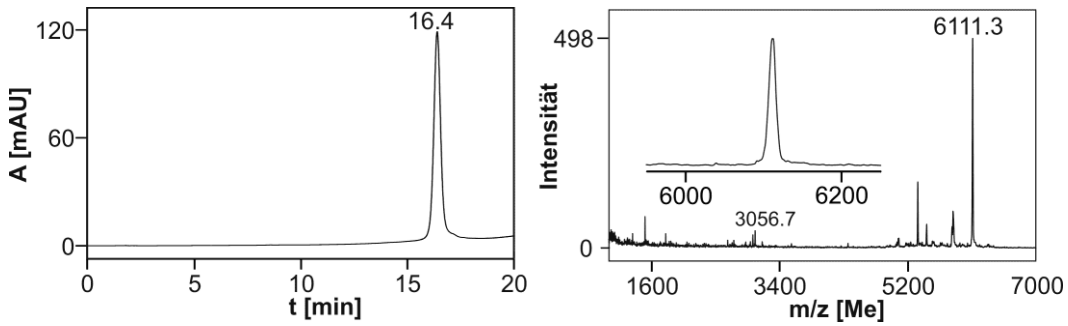


Figure S24 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **DY752-13**.

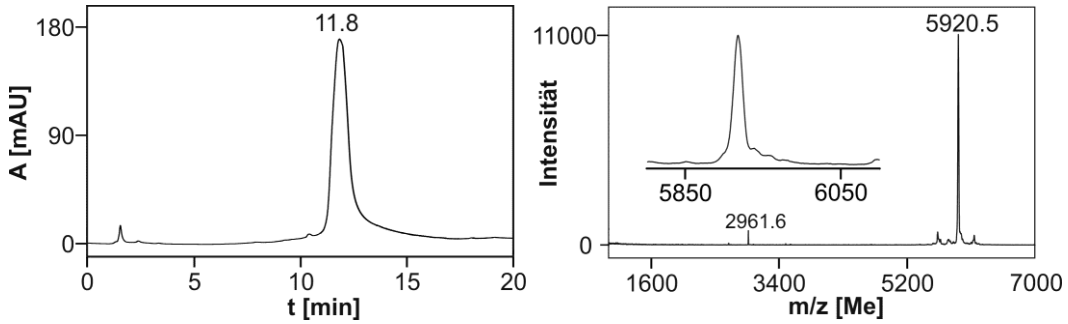


Figure S25 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **ITCC-4**.

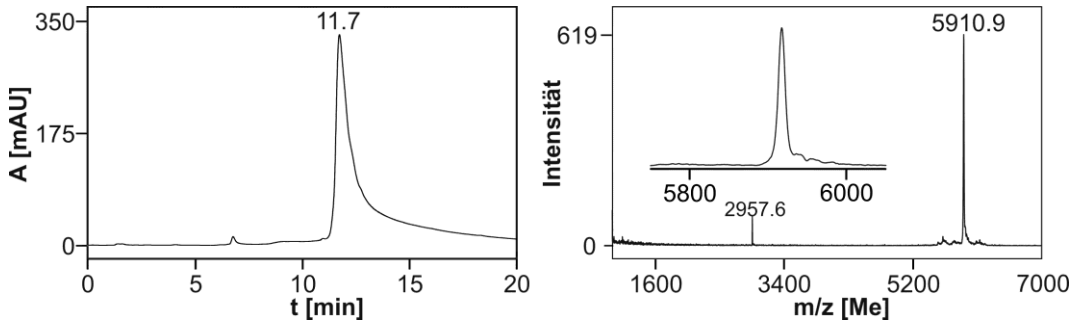


Figure S26 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **ITCC-7**.

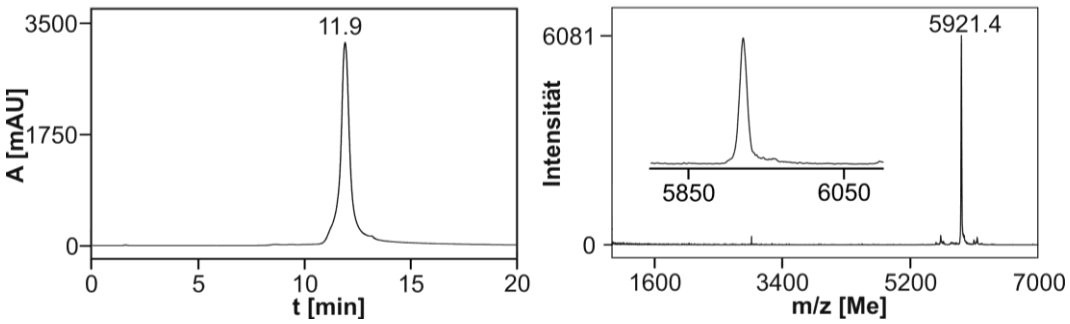


Figure S27 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **ITCC-10**.

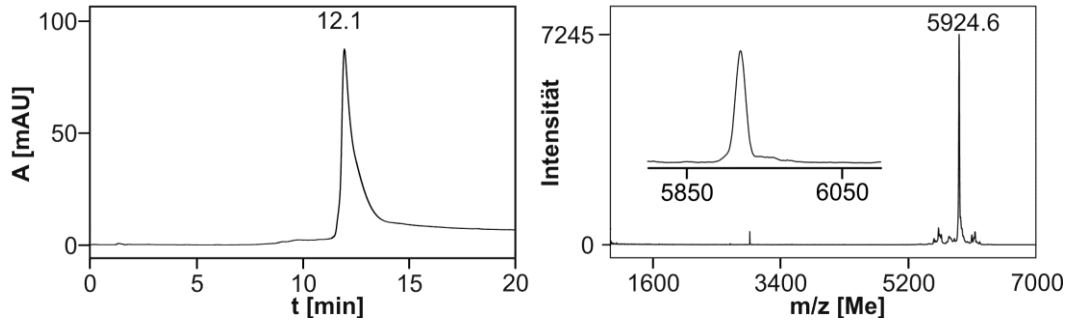


Figure S28 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **ITCC-13**.

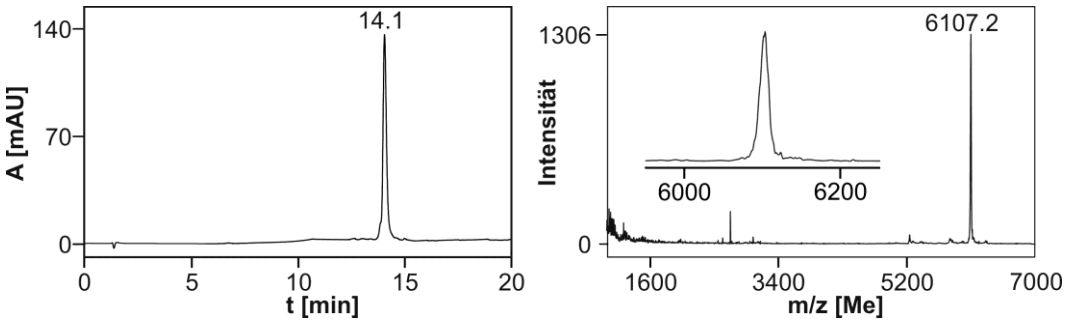


Figure S29 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **NIR797-4**.

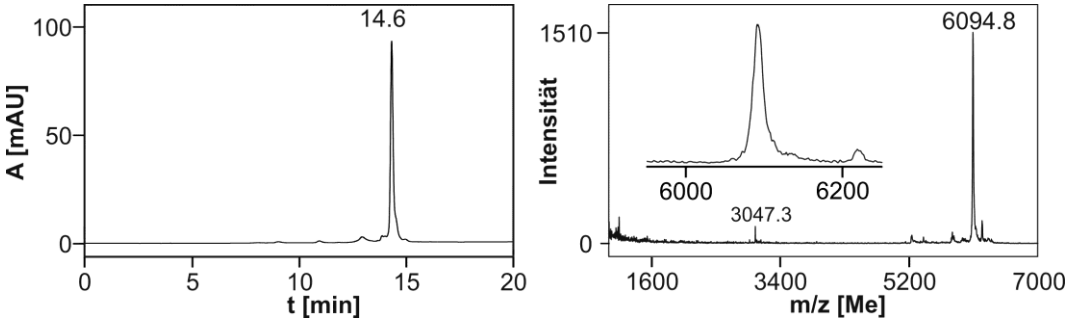


Figure S30 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **NIR797-7**.

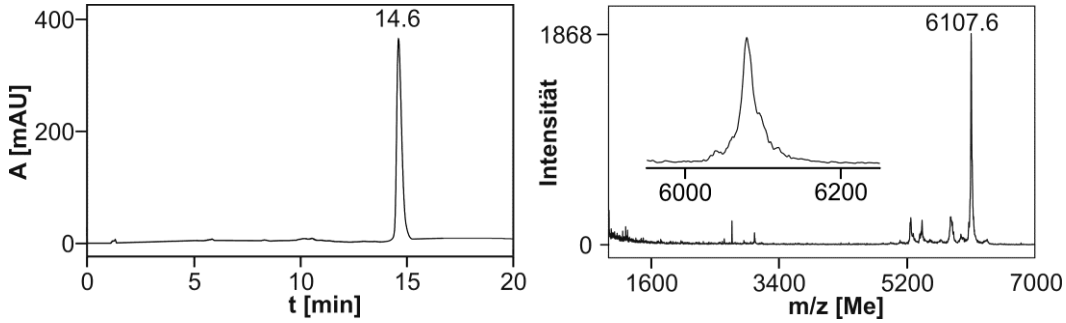


Figure S31 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **NIR797-10**.

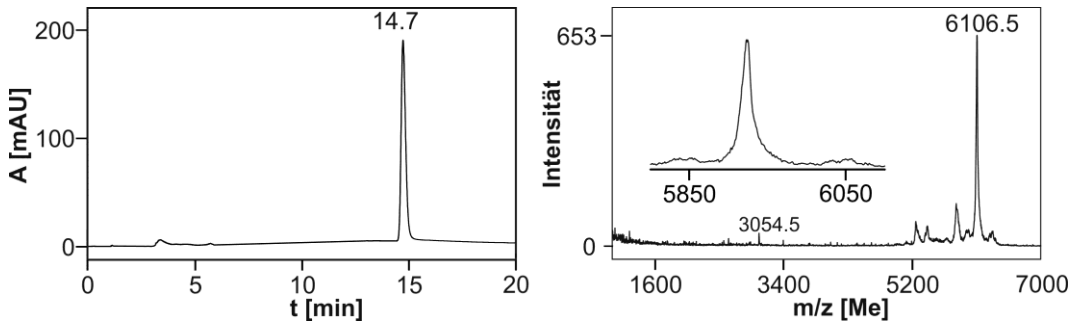


Figure S32 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **NIR797-13**.

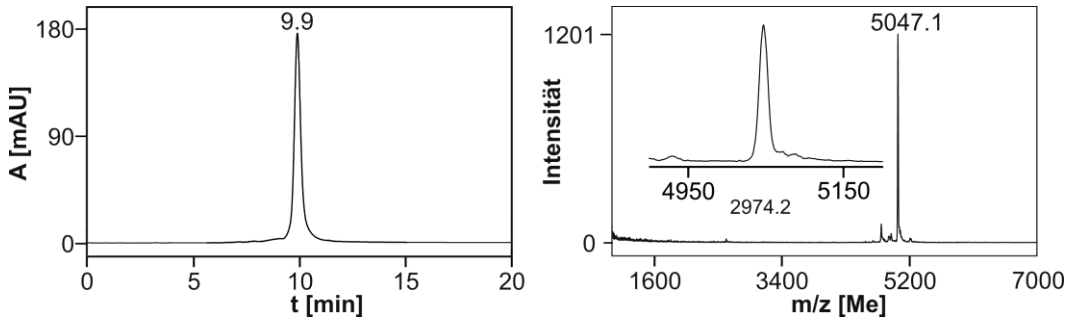


Figure S33 HPLC trace (Gradient I) and MALDI-TOF mass spectrum of probe **NA**.

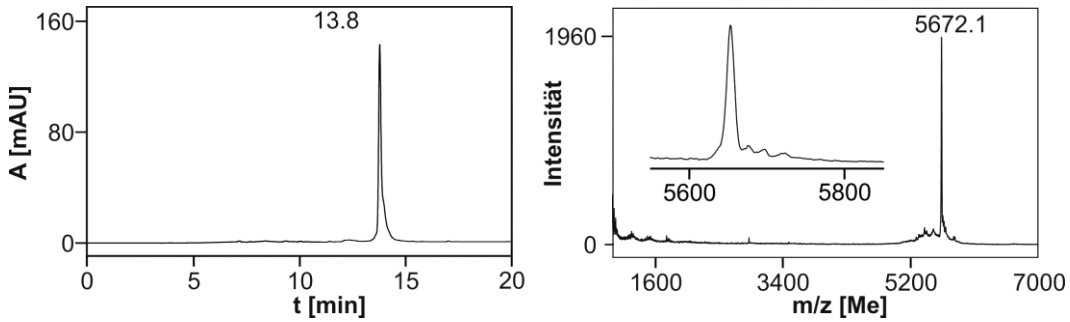


Figure S34 HPLC trace (Gradient I) and MALDI-TOF mass spectrum of probe **ICC-NA10**.

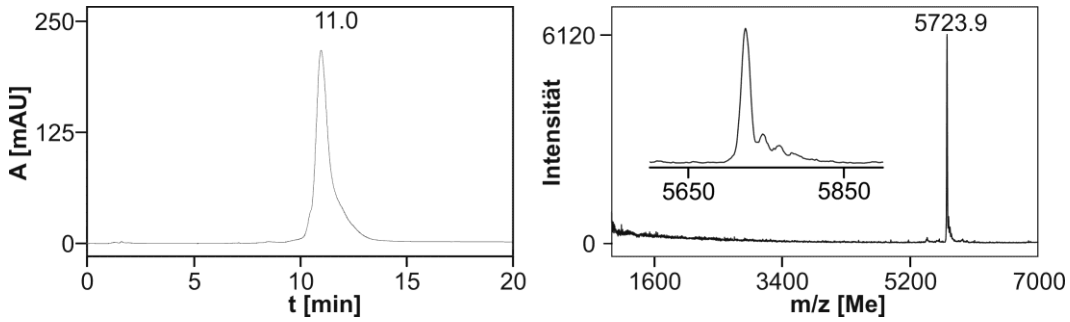


Figure S35 HPLC trace (Gradient III) and MALDI-TOF mass spectrum of probe **ITCC-NA10**.

3. T_M measurements

T_M measurements were carried out by measuring the absorption at 260 nm by using a Varian Cary 100 Bio-UV/Vis spectrometer in 100µl ultra-micro fluorescence cuvettes 105.250-QS from Hellma upon thermal denaturation. The solution was covered with 200 µl of mineral oil at 1 µM concentration in a buffered solution (100 mM NaCl, 10 mM NaH_2PO_4, pH 7). Melting curve program: 95°C for 1 min, 45°C for 1 min, followed by a temperature ramp from 55 to 95°C with a slope of 0.1°C/sec. Melting temperatures (T_M) were calculated from the first derivatives.

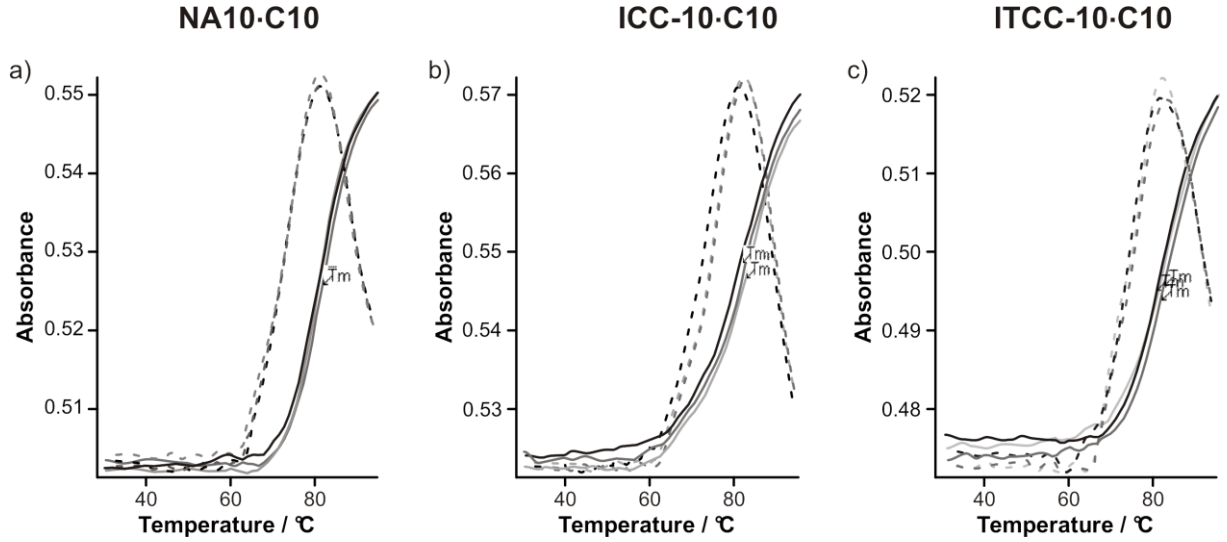


Fig. S36 Representative melting curves

4. Fluorescence spectra

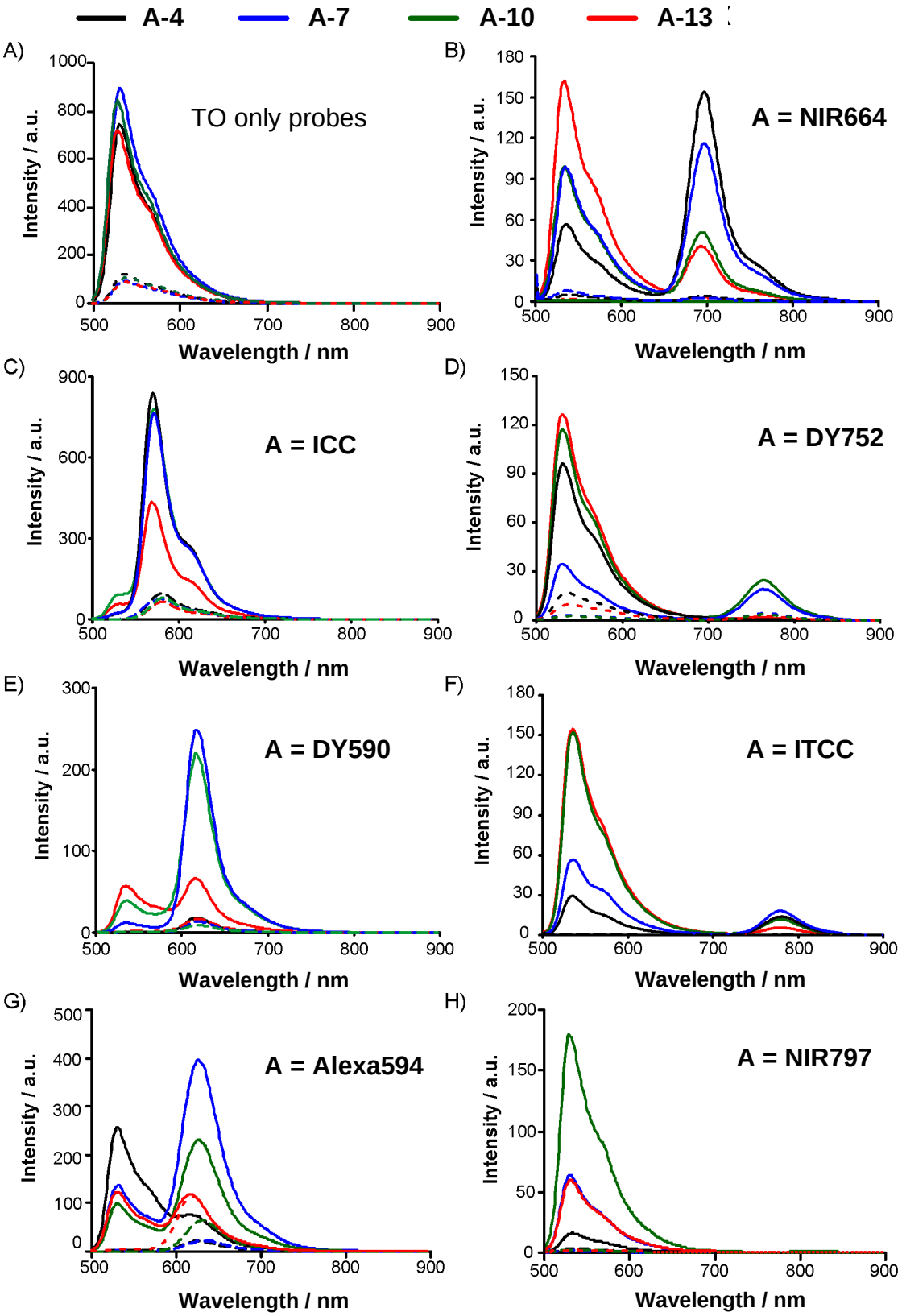


Figure S37 Fluorescence spectra of probes before and after additions of matched DNA. Conditions: 1 µM probe and target in 100 mM NaCl, 10 mM NaH_2PO_4, pH 7.0 at 25°C, λ_{ex} = 485 nm.

5. UV Spectra

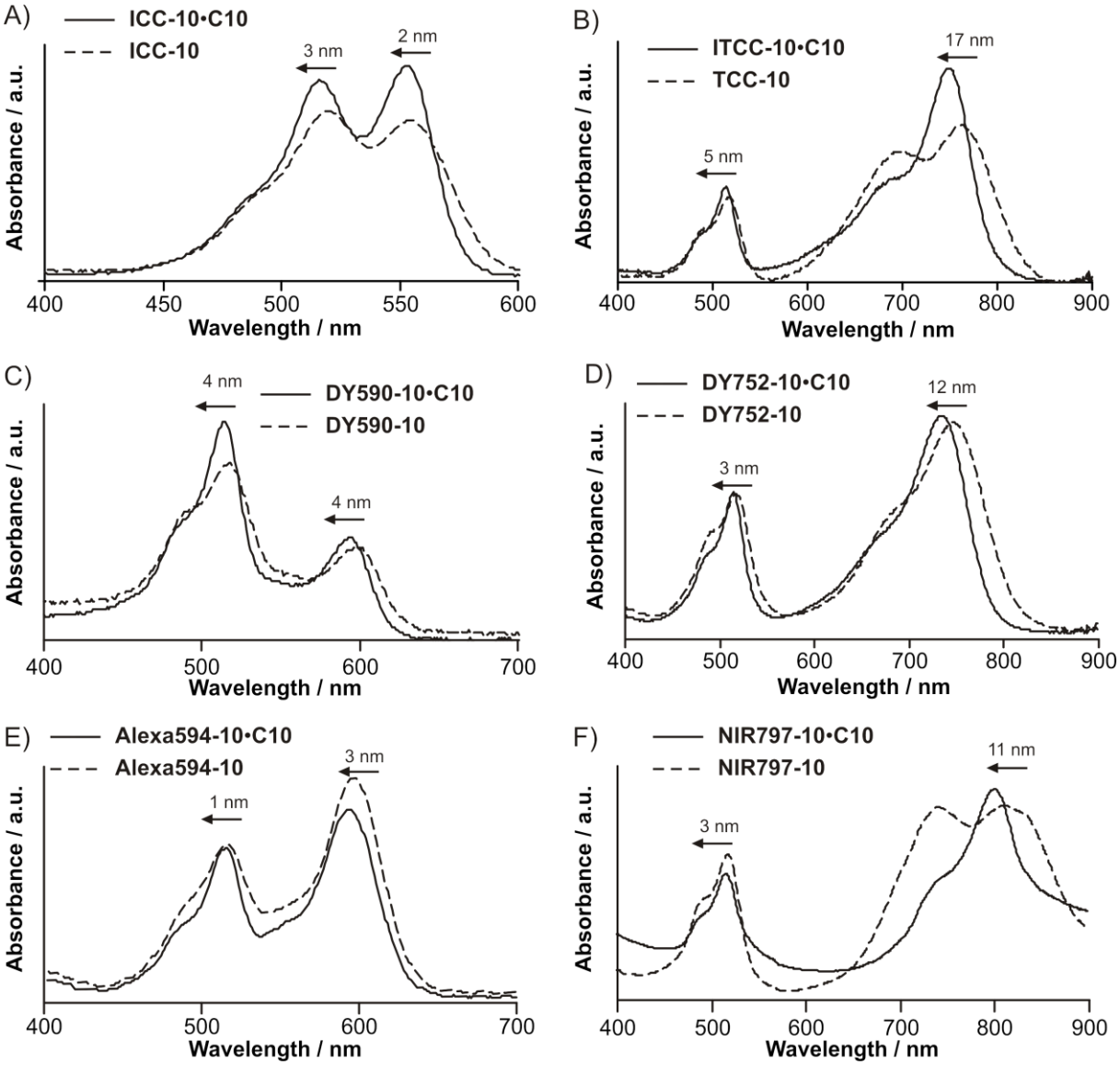


Figure S38 UV spectra of probes before and after addition of matched DNA **C10**. Conditions: 1 µM probe and target in 100 mM NaCl, 10 mM NaH_2PO_4, pH 7.0 at 25°C. Large hypsochromic shifts (> 10 nM) of acceptor absorption upon hybridization is probably due to disruption of donor-acceptor contact.

6. Temperature Dependence

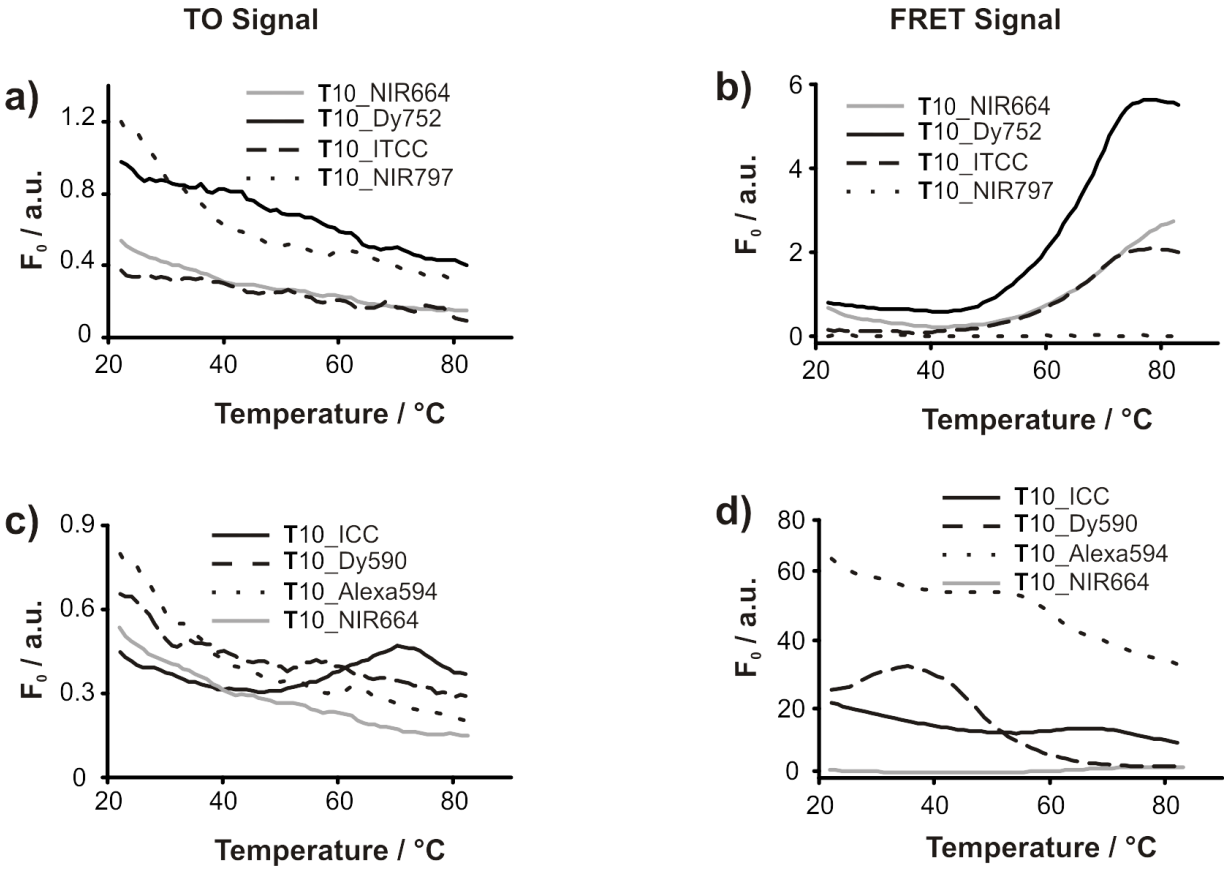


Figure S39 Temperature dependence of background fluorescence F_0 of a) and c) TO emission and of b), c) FRET emission. Emission in a) and c) was measured at maximum of donor fluorescence (TO signal) and in b) and d) at the maximum of the acceptor dye fluorescence (FRET signal).

7. Excitation Spectra

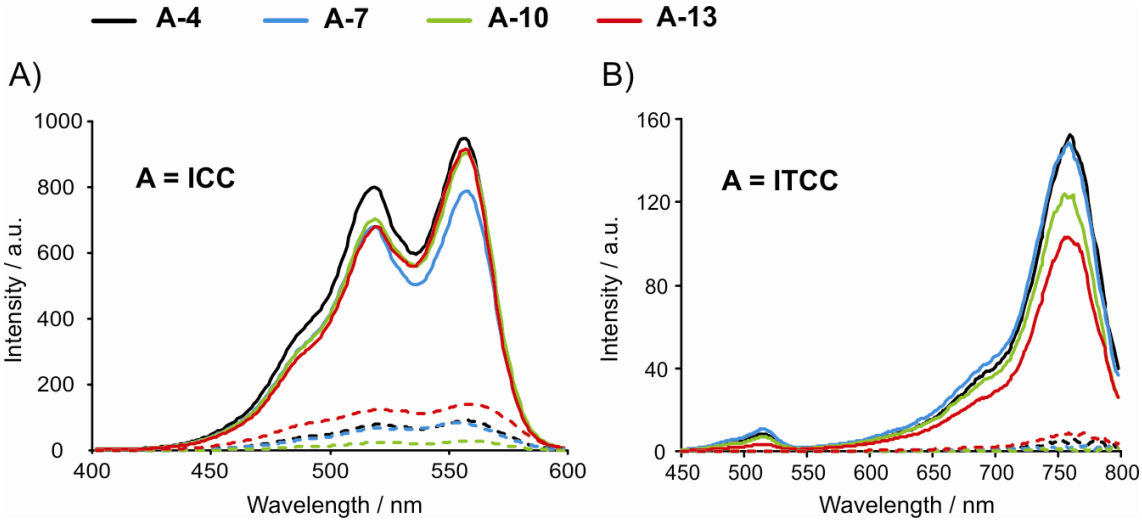


Figure S40 Excitation spectra of probes before (dashed) and after (full line) addition of matched DNA at emission wavelength of A) λ_{em} = 620 nm and B) λ_{em} = 820 nm. Conditions: 1 µM probe and target in 100 mM NaCl, 10 mM NaH_2PO_4, pH 7.0 at 25°C,