

Supplementary Methods

Reagents

Unless otherwise denoted, chemical compounds and proteins were from Sigma-Aldrich-Fluka (Buchs, Switzerland)

Synthesis of ESAC compounds

Activated derivatives of the lead binders to HSA and CA were coupled to the 24mer amino-modified oligonucleotides 5'-CAGCACACAGAATTCAGAAGCTCC-3', carrying a primary amino group linked to the N⁴ of the 3'-terminal cytosine (IBA; Cat.No. 5-0220-014), as follows:

Lead HSA binder: 10 nmol (40 µl) of the above mentioned 24mer 3' amino-modified oligonucleotide in 0.25 M sodium carbonate buffer, pH 9.0, were reacted over night at room temperature with the same volume of a 5 mM solution of dansyl chloride (Sigma, Prod. Nr. D2625) (0.2 µmol) in acetonitrile. Excess of dansyl chloride was quenched by addition of 0.5 µmol of ethanolamine. The reaction was purified by HPLC on a LUNA C18(2) column (Phenomenex, Torrance CA, USA) using a linear gradient from 100 mM triethylammonium acetate (TEAA), pH 7.0 to 100 mM TEAA containing 80% (v/v) acetonitrile, pH 7.0. Solvents and volatile salt were removed under vacuum. MALDI-MS was performed as described below.

Lead CA binder: 5 nmol (40 µl) of the above mentioned 24mer 3' amino-modified oligonucleotide in 62.5 mM sodium carbonate buffer, pH 9.0, were reacted over night at room temperature with the same volume of a 2.5 mM solution of 4-sulfamidobenzoyl chloride (DMF complex), Prod.Nr. 18428, Lancaster Synthesis, Newgate, Lancashire, UK) (0.1 µmol) in acetonitrile. Excess of dansyl chloride was quenched by addition of 0.25 µmol of ethanolamine. The reaction was purified as mentioned above (by HPLC on a LUNA C18(2)). Solvents and volatile salt were removed under vacuum.

MALDI-TOF spectra of the initial protein binders were measured on a Voyager-DE Elite Instrument (Applied Biosystems) in reflector mode. Samples were directly spotted onto the target plate (matrix: 3-hydroxypicolinic acid) according to ZipTip_{C18}[®] Technical Note TN225 (Millipore, Bedford MA, USA). (3' amino-modified 24mer oligonucleotide: m/z = 7799; lead HSA binder: m/z = 8033; lead CA binder: m/z = 8039)

The individual organic compounds to be coupled to the 5' amino-modified 48-mer oligonucleotides were dissolved in DMSO, occasionally by further addition of water or diluted hydrochloric acid.

Coupling reactions of carboxylic acids: To a reaction volume of 100 µl final, containing 50% (v/v) DMSO, compounds were added to the respective final concentrations: DMSO-dissolved carboxylic acid, 25 mM; N-hydroxysulfosuccinimide (Fluka, Buchs, Switzerland), 25 mM; N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (Fluka), 5 mM; sodium carbonate buffer, pH 9.0, 150 mM; oligonucleotide, 100 µM.

Coupling reactions of N-hydroxysuccinimidyl esters: To a reaction volume of 100 µl final, containing 50% (v/v) DMSO, compounds were added to the respective final concentrations: N-hydroxysuccinimidyl esters, 1mM; sodium carbonate buffer, pH 9.0, 30 mM; oligonucleotide, 100 µM.

Coupling reactions of isothiocyanates: To a reaction volume of 100 µl final, containing 50% (v/v) DMSO, compounds were added to the respective final concentrations: isothiocyanates, 0.5 mM; sodium carbonate buffer, pH 8.5, 30 mM; oligonucleotide, 100 µM.

All coupling reactions were stirred over night at room temperature, residual activated species were then quenched by addition of Tris-Cl buffer, pH 8.0. Oligonucleotide in each reaction mixture was allowed to quantitatively precipitate by sequential addition of 25 µl of 1 M acetic acid, 12.5 µl of 3 M sodium acetate buffer, pH 4.7 and 500 µl ethanol followed by 30 min incubation on ice. The mixture was centrifuged and the resulting oligonucleotide pellet was washed with cold 70% (v/v) ethanol and then dissolved in 100 µl millipore water. The reactions of compounds #31, 76, 81, 92, 94 which co-precipitated with the oligonucleotide and oligonucleotide #123 were excluded from the library. Typical coupling yields were >75%.

Test coupling reactions were also performed with a 5mer 5' amino-modified oligonucleotide (for which high-precision MALDI-TOF measurements are possible) using biotin as model carboxylic acid or biotin-NHS as activated ester, using the reaction conditions described above. The reactions were then purified by HPLC on a LUNA C18(2) column (Phenomenex) as described above. The masses of the reacted oligonucleotides showed the expected product of mono-biotinylation ($m/z = 1916.6$ and 1915.2 , respectively, compared to $m/z = 1689.0$ for the 5mer 5' amino-modified test oligonucleotide).

A list of oligonucleotide codes and chemical compounds used to construct an ESAC library can be found in the *Supplementary Table 1*.

Synthesis of bidentate ligands

Initial binders of HSA and CA were linked to compounds derived from ESAC selections using three different diamino linkers (FLUKA, Buchs, Switzerland): 1) 1,6-diaminohexane; 2) 1,8-diaminooctane; 3) 1,4-Bis(3-aminopropoxy)butane.

HSA binders were synthesized in a two step reaction as follows:

Dansyl chloride (Prod.Nr. D2625, Sigma, Buchs, Switzerland) (final concentration 33.3 mM) was reacted overnight at room temperature with the diamino linker (final concentration 333.3 mM) in DMF. Excess of dansyl chloride was quenched by addition of ethanolamine. Monoalkylated diamino linker was purified from the reaction mixture by HPLC on a LUNA C18(2) column (Phenomenex), using a linear gradient from 100 mM TEAA, pH 7.0 to 100mM TEAA containing 80% (v/v) acetonitrile, pH 7.0. In a second step, the monoalkylated amines were coupled to the selected compounds C17 and C37, respectively, as follows: N-Succinimidyl-7-(diethylamino)coumarin-3-carboxylate (C17-NHS) (Prod. Nr. 36801, FLUKA, Buchs, Switzerland)(20 mM) was reacted over night at room temperature with the respective monoalkylated diamine (40 mM) in sodium carbonate buffer, pH 9.0, containing 50% (v/v) DMF. Precipitated compound (in the case of 1,6-diaminohexane and 1.8-diaminooctane) was dissolved and residual activated ester was quenched by addition of Tris buffer. The desired dialkylated compounds were then purified by HPLC as described above. ESI-MS: dansylamide-1,6-diaminohexane-C17: $m/z = 593.1$ ($[M+H]^+$); dansylamide-1,4-Bis(3-aminopropoxy)butane-C17: $m/z = 702.9$ ($[M+Na]^+$).

Suberic acid monomethylester (C37) (Prod. Nr. 242446, Aldrich) (f.c. = 15 mM) was reacted over night at room temperature with a mixture of the respective monoalkylated diamine (7.2 mM), EDC (7.5mM), sulfo-NHS (8.3mM) in sodium carbonate buffer, pH 9.0 (50mM), containing 30% (v/v) DMSO). Residual activated ester was quenched by addition of ethanolamine and the desired dialkylated compounds were then purified by HPLC as described above. ESI-MS: dansylamide-1,6-diaminohexane-C37: $m/z = 520.2$ ($[M+H]^+$); dansylamide-1,8-diaminooctane-C37: $m/z = 548.2$ ($[M+H]^+$); dansylamide-1,4-Bis(3-aminopropoxy)butane- C37: $m/z = 608.1$ ($[M+H]^+$).

CA binders were synthesized in a two step reaction as follows:

4-sulfamidobenzoyl chloride (DMF complex, Prod.Nr. 18428, Lancaster Synthesis, Newgate, Lancashire, UK) (final concentration 33.3 mM) was reacted over night at room temperature with the diamino linker 1.8-diaminooctane (final concentration 333.3 mM) in DMF. Precipitated compound was dissolved and excess of 4-sulfamidobenzoyl chloride was quenched by addition Tris buffer. Monoalkylated diamino linker was purified from the reaction mixture by HPLC on a LUNA C18(2) column (Phenomenex) using a linear gradient from 100 mM TEAA, pH 7.0 to 100 mM TEAA containing 80% (v/v) acetonitrile, pH 7.0. ESI-MS: $[M+H]^+$: $m/z = 328.2$. In a second step, the monoalkylated amine was coupled to the selected compounds C10 (Theophylline-7-acetic acid; Prod. Nr. 88310, Fluka) and C60 (Betain hydrochloride; Prod. Nr. 147931, Aldrich) respectively, according to a general

procedure: The compound (32.3 mM final concentration) was reacted over night at room temperature with a mixture of the respective monoalkylated diamine (3.2 mM final concentration), EDC (9.7 mM final concentration), sulfo-NHS (16.1 mM final concentration) in sodium carbonate buffer, pH 9.0 (100 mM), containing 50% (v/v) DMSO). Residual activated ester was quenched by addition of Tris buffer and the desired dialkylated compounds were then purified by HPLC as described above. ESI-MS: 4-sulfamidobenzamide-1,8-diaminooctane-C10: $m/z = 570.1$ ($[M+Na]^+$); 4-sulfamidobenzamide-1,8-diaminooctane-C60: $m/z = 429.1$ ($[M+H]^+$).