



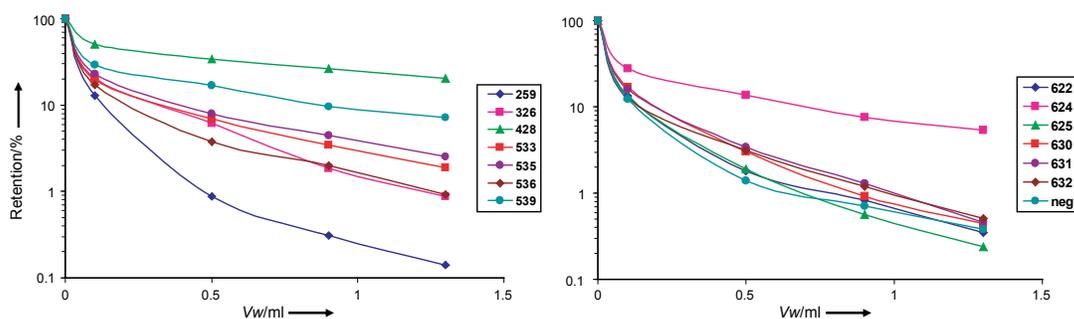
Supporting Information

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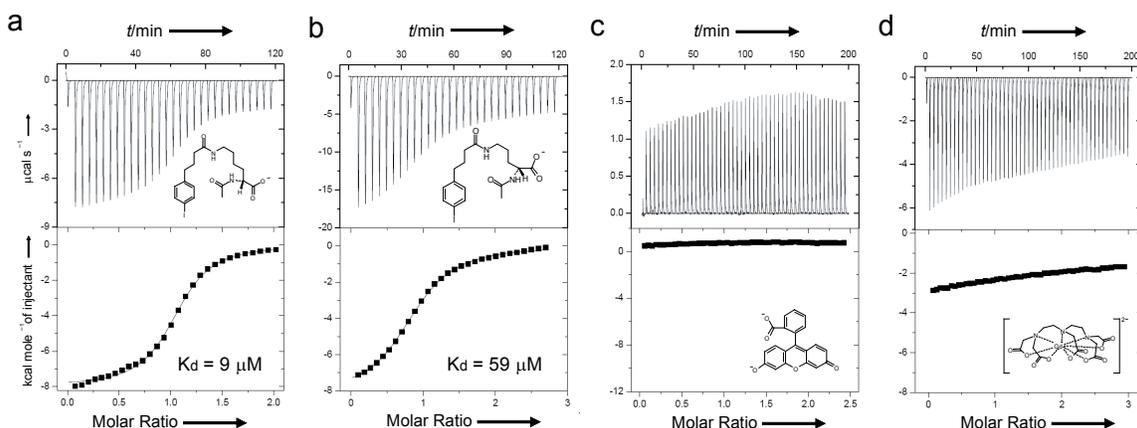
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# Discovery and Applications of a Portable Albumin Binder from a DNA-Encoded Chemical Library

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Supplementary Figure 1: Primary analysis of identified binding molecules. Chromatographic albumin binding assay of the oligonucleotide-compound conjugates and an unmodified oligonucleotide (neg) paired with a  $^{33}\text{P}$  labeled DNA strand to resin presenting HSA. The percentage of oligonucleotide-compound conjugate retained on the resin is plotted vs. the washing volume (Vw).



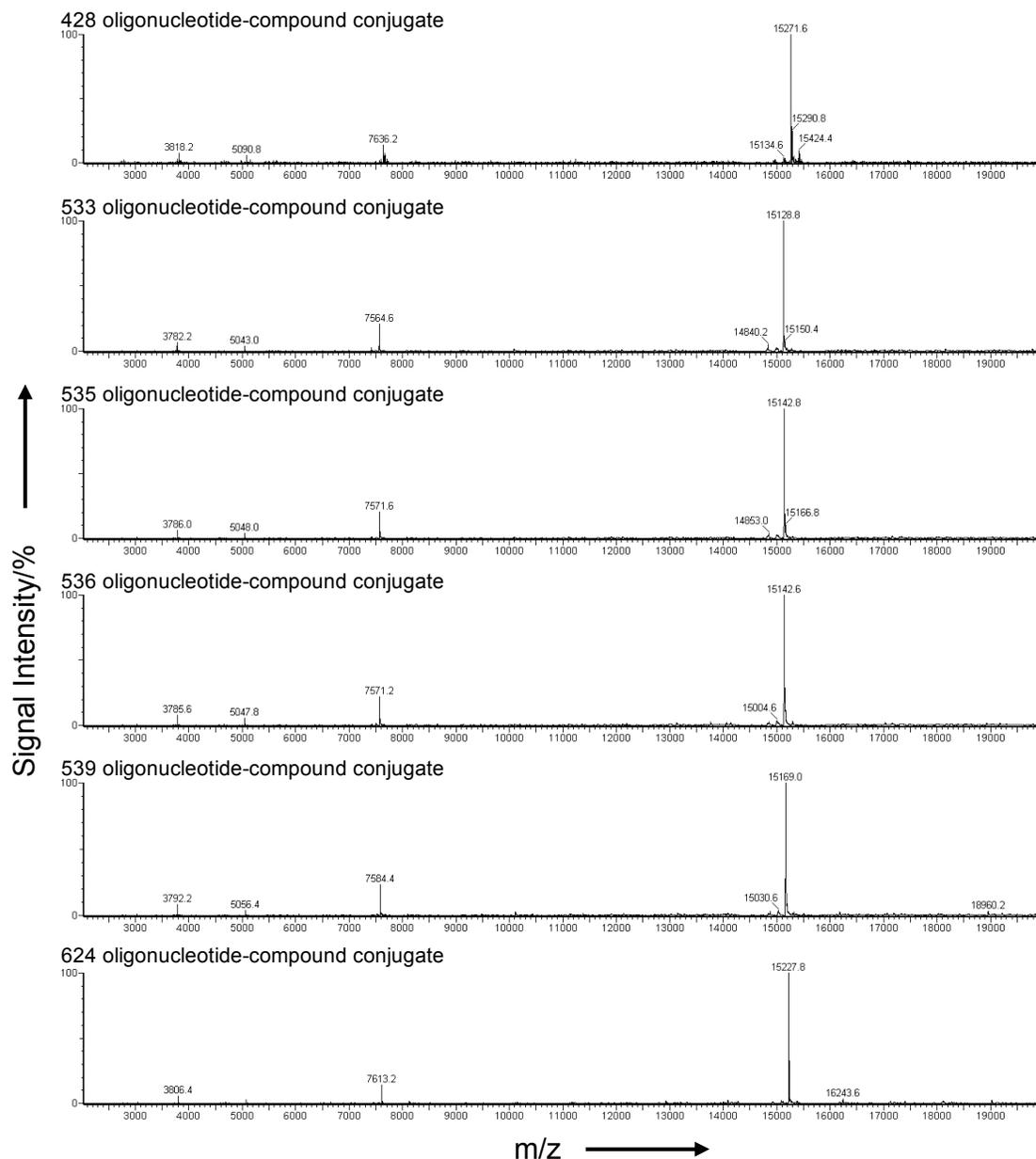
Supplementary Figure 2: ITC profile of HSA titrated with **428-L-Lys-Ac** (a), **428-L-Orn-Ac** (b), fluorescein (c), and Gd-DTPA (d) at 37 °C. The affinity constants and the structures of the molecules and where possible the affinity constants are depicted.

Inhibitor	Binding site	1 µM	10 µM	100 µM
Warfarin	I	147	124	138
Phenylbutazone	I	130	146	113
Indomethacin	I	141	101	59
Ibuprofen	II	128	91	65
Dansylsarcosine	II	132	98	72
Diazepam	II	142	118	81
L-Tryptophane	II	144	143	132
Myristic acid	Fatty acids	103	42	31
CuSO <sub>4</sub>	Metal ion	137	143	135

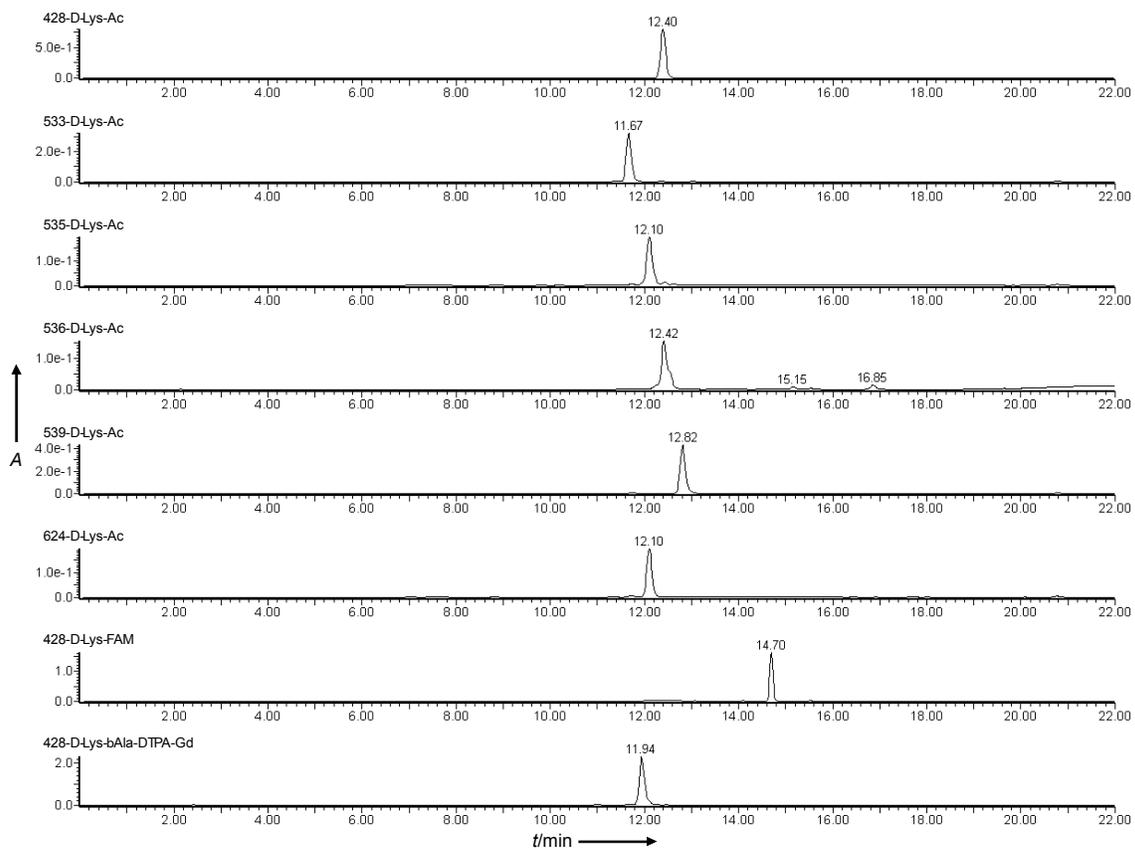
Controls	FP
No competitor	144
No HSA	33

Supplementary Figure 3: Fluorescence polarization of competition for **428-D-Lys-FAM** binding to HSA. 500 nM of **428-D-Lys-FAM** and HSA were incubated with increasing amounts of competitor (left panel). The control values for unbound (no HSA) and bound (no competitor) **428-D-Lys-FAM** are given (right panel). These results indicate binding of **428-D-Lys** at site II. Competition with fatty acids is in consistence with site II binding because the fatty acid binding sites 3 and 4 are

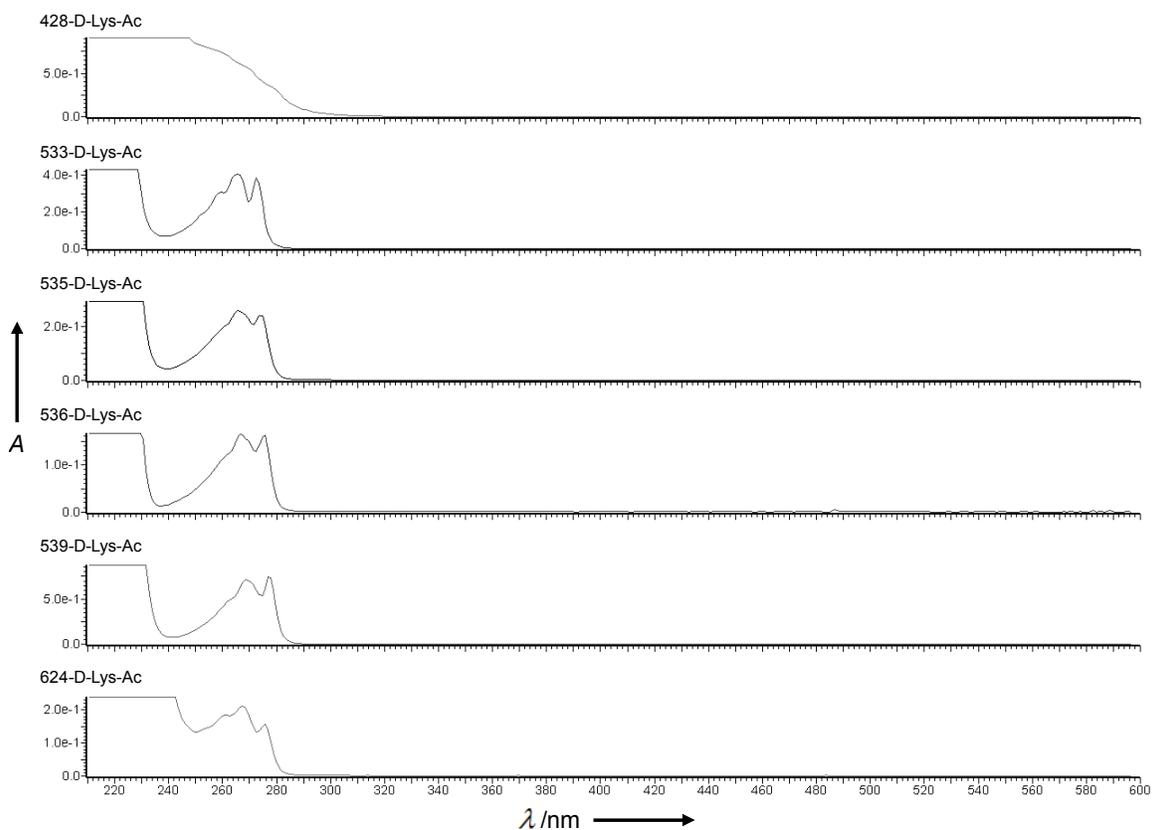
partially overlaying with site II<sup>[1]</sup>. In addition many site I ligands are known to bind to site II with lower affinity explaining the competition with indomethacin.



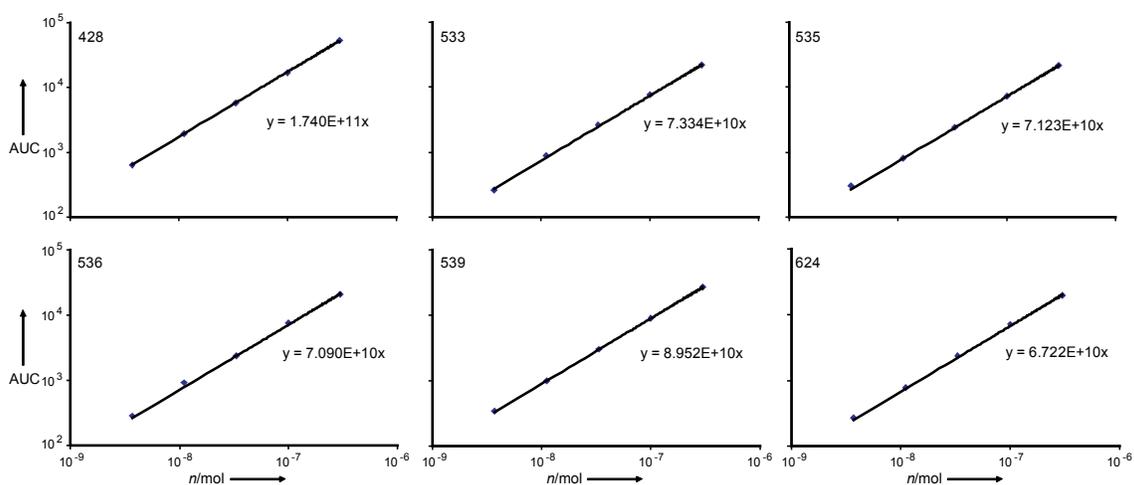
Supplementary Figure 4: ESI-MS analysis of the oligonucleotide-compound conjugates enriched in the selection for binding to HSA. The molecular weights of the uncharged conjugates are 15274.9 Da (**428**), 15132.0 Da (**533**), 15146.0 Da (**535**), 15146.0 Da (**536**), 15172.1 Da (**539**), and 15227.9 Da (**624**).



Supplementary Figure 5: HPLC chromatogram at 260 nm of the molecules studied.



Supplementary Figure 6: Absorption spectra of the enriched compounds conjugated to acetylated D-lysine.



Supplementary Figure 7: Plot of the area under the curve (AUC) vs. the molar amount of the enriched compounds analyzed by HPLC-UV at 260 nm. The fitted curves are indicated.

## Methods

Unless stated otherwise all materials were purchased from Sigma-Aldrich.

### Selection

The single stranded DNA-encoded chemical library consisted of 619 individual compounds conjugated to the 5' end of 48mer oligonucleotides of the general format 5'-NH<sub>2</sub>(CH<sub>2</sub>)<sub>6</sub>PO<sub>4</sub>-GGA GCT TCT GAA TTCTGT GTG CTG XXX XXX CGA GTC CCA TGG CGC AGC-3' (where XXX XXX denotes different unique combinations of six bases for the individual compounds, with three/six bases being G or C) synthesized and characterized as described previously<sup>[2]</sup>. The mass spectrometric analyses of the oligonucleotide-compound conjugates enriched in the selection are given in Supplementary Figure 4. The library containing each member at a concentration of 1.6 nM was dimerized by heteroduplex formation with an unmodified pairing oligonucleotide at a concentration of 100 nM. The resulting DNA duplex was diluted 1:10 in phosphate buffered saline (PBS), and 100 μL were added to 50 μL of HSA modified or inactivated resin preincubated with PBS, 0.3 mg mL<sup>-1</sup> herring sperm DNA. After incubation for 1 h at 25 °C, the suspension was transferred to a SpinX column (Corning Incorporated), the supernatant removed, and the resin washed 3 x with 300 μL of PBS, 1 mM MgCl<sub>2</sub>, 0.1% Tween 20. The resin was resuspended in 100 μL of H<sub>2</sub>O.

Inactivated and HSA resin were prepared as follows: 150 mg of CNBr-Sepharose (GE Healthcare) were resuspended and excessively washed with cold 1 mM HCl. 500 μl of 100 mM Tris-HCl pH 8 or 140 mg ml<sup>-1</sup> HSA in 100 mM NaHCO<sub>3</sub>, 100 mM NaCl, pH 8.9 were added and incubated at 4 °C overnight. After reaction excess reactive groups on the beads were quenched using 100 mM Tris-HCl, pH 8, and the beads were washed repeatedly with alternating buffers (0.1 M sodium

acetate, 0.5 M NaCl, pH 4, and 0.1 M Tris-HCl, 0.5 M NaCl, pH 8) using SpinX columns (Corning Incorporated).

### Decoding

The codes of the oligonucleotide-compound conjugates were amplified after selection by PCR (50  $\mu$ L, 25 cycles of 1 min at 94 °C, 1 min at 55 °C, 40 s at 72 °C) of 5  $\mu$ L of the resuspended resin using primers AB\_fo\_short (GGA GCT TCT GAA TTC TGT GTG CTG) and Elib2\_ba (GCT GCG CCA TGG GAC TCG). The product was purified with a PCR purification kit (QIAGEN) and subsequently linearly amplified by PCR (see above) using 5'-Cy3-labeled Elib2\_ba as primer. The product was precipitated by adding 10% v/v of 3 M NaOAc, pH 4.7, and 250% v/v of ethanol and redissolved in 120  $\mu$ L of hybridization buffer (4 x sodium chloride – sodium citrate (SSC), 50 mM HEPES, 0.2% SDS, pH 7) and incubated with microarray glass slides (displaying 19mers with the structure 5'-TGTGCTGXXXXXXCGAGTC-3' in quintuplicate) in a Tecan HS 400 hybridization instrument for 4 h at 44 °C followed by successive washing steps with 2 x SSC/0.2% (w/v) SDS, 0.2 x SSC/0.2% (w/v) SDS, and 0.2 x SSC. After hybridization, microarrays were analyzed by using a Genepix professional 4200A scanner ( $\lambda_{\text{ex}} = 532$  nm, 100% laser power, photomultiplier 300). Spot intensities were quantified and evaluated using Genespotter Software (v2.4.3). After background subtraction, the mean value was used as spot signal intensity (average of five spots). Enriched binding molecules were identified by the ratio of the geometric mean of two selections against HSA and inactivated resin.

### Chromatographic Albumin Binding Assay

A 24mer oligonucleotide with a complementary sequence to the hybridization domain of the library oligonucleotides was radioactively labeled at the 5'-terminus using  $\gamma^{33}\text{P}$ -ATP (GE Healthcare) and T4 polynucleotide kinase (USB). In separate tubes the radiolabeled oligonucleotide was hybridized to one of the

selected oligonucleotide-compound conjugates or an unmodified oligonucleotide serving as negative control at an oligonucleotide concentration of 100 nM each. Aliquots of these oligonucleotide duplex pairs were incubated with HSA resin in 100  $\mu$ l of PBS as described above. After 1 h of incubation, three rounds of washing were performed with 400  $\mu$ l of PBS, and the beads as well as aliquots of the input, flow-through, and all washing fractions were subjected to  $^{33}\text{P}$  radioactivity counting with a Beckman LS 6500 scintillation counter.

### Synthesis of compounds

**428**-aminoheptanoic acid: 10  $\mu$ mol of **428** were stirred with 8  $\mu$ mol of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) and 10  $\mu$ mol of *N*-hydroxysuccinimide (NHS) in 50  $\mu$ l of DMSO for 3 hours at 30 °C followed by addition of 20  $\mu$ mol of 7-aminoheptanoic acid (Bachem) in 100  $\mu$ l of 1 M  $\text{NaHCO}_3$ , pH 9. The reaction was allowed to stir overnight at 30 °C. After quenching of remaining reagents with excess of Trizma base the reaction was purified by HPLC.

X-D-Lys-Ac (X = **326**, **428**, **533**, **535**, **536**, **539**, **622**, and **624**): Activation of the carboxylic acid was performed as described above. After addition of 20  $\mu$ mol of Fmoc-D-Lys (Bachem) and 43  $\mu$ mol of triethylamine (TEA) in 100  $\mu$ l DMSO the reaction was stirred overnight at 30 °C. Removal of the Fmoc-protection group at the  $\alpha$ -amino position was performed by addition of 10  $\mu$ l of 5 M KOH and stirring for 2 hours at 30 °C. After purification by HPLC on a 0.1% trifluoroacetic acid gradient, the dried fractions containing X-D-Lys were resuspended in DMSO. 5 M HCl was added until the solution became clear. X-D-Lys was reacted with 10x molar excess of  $\text{Ac}_2\text{O}$  for 4 hours at 30 °C after concentration determination by HPLC-UV. The product was purified by HPLC.

FAM-derivatives: 19  $\mu$ mol of phenethylamine, **428**-D-Lys, or **622**-D-Lys prepared as above and 14  $\mu$ mol of 5-carboxyfluorescein NHS ester were dissolved in 120

$\mu\text{l}$  DMSO. 72  $\mu\text{mol}$  of TEA were added. The reaction was stirred protected from light for 24 hours at 30 °C. The reaction was purified by HPLC.

**428-D-Lys- $\beta$ -Ala-DTPA-Gd:** Fmoc- $\beta$ -Ala was conjugated to **428-D-Lys** with EDC / NHS, deprotected with 5 M KOH and purified as described above. 19  $\mu\text{mol}$  of **428-D-Lys- $\beta$ -Ala** and 14  $\mu\text{mol}$  of p-SCN-Bn-DTPA (Macrocyclics) were dissolved in 120  $\mu\text{l}$  DMSO. 72  $\mu\text{mol}$  of TEA were added. The reaction was stirred for 24 hours at 30 °C. The reaction was purified by HPLC. Complex formation between **428-D-Lys- $\beta$ -Ala-DTPA** and  $\text{Gd}^{3+}$  was performed by adding 10  $\mu\text{mol}$  of  $\text{GdCl}_3$  to 1  $\mu\text{mol}$  **428-D-Lys- $\beta$ -Ala-DTPA** and adjusting to pH 10 with NaOH, followed by overnight incubation at 25 °C. Removal of excess  $\text{Gd}^{3+}$  was performed by HPLC on a triethylammonium acetate (TEAA) gradient.

**428-D-Lys-Ac:**  $^1\text{H-NMR}$  (400 MHz, MeOD)  $\delta$  7.62 (d,  $J$  = 8.2 Hz, 2H), 7 (d,  $J$  = 8.2 Hz, 2H), 4.38-4.34 (m, 1H), 3.18 (t,  $J$  = 6.69 Hz, 2H), 2.59 (t,  $J$  = 7.45 Hz, 2H), 2.19 (t,  $J$  = 7.45 Hz, 2H), 1.99 (s, 3H), 1.94-1.83 (m, 3H), 1.76-1.67 (m, 1H), 1.58-1.48 (m, 2H), 1.46-1.39 (m, 2H). ESI-MS ( $m/z$ ): 460.85 ( $[\text{M}+\text{H}^+]$ , 100%), calc. 460.09.

**533-D-Lys-Ac:**  $^1\text{H-NMR}$  (400 MHz, MeOD)  $\delta$  7.08 (m, 4H), 4.38-4.35 (m, 1H), 3.18 (t,  $J$  = 7.04 Hz, 2H), 2.59 (t,  $J$  = 7.54 Hz, 2H), 2.3 (s, 3H), 2.19 (t,  $J$  = 7.93 Hz, 2H), 1.99 (s, 3H), 1.94-1.83 (m, 3H), 1.76-1.67 (m, 1H), 1.58-1.38 (m, 4H). ESI-MS ( $m/z$ ): 348.94 ( $[\text{M}+\text{H}^+]$ , 100%), calc. 348.2.

**535-D-Lys-Ac:**  $^1\text{H-NMR}$  (400 MHz, MeOD)  $\delta$  7.02 (d,  $J$  = 7.68 Hz, 1H), 6.96 (s, 1H), 6.9 (d,  $J$  = 7.68 Hz, 1H), 4.38-4.35 (m, 1H), 3.18 (t,  $J$  = 6.94 Hz, 2H), 2.56 (t,  $J$  = 7.68 Hz, 2H), 2.24 (s, 3H), 2.22 (s, 3H), 2.18 (t,  $J$  = 7.44 Hz, 2H), 1.98 (s, 3H), 1.93-1.83 (m, 3H), 1.76-1.66 (m, 1H), 1.58-1.38 (m, 4H). ESI-MS ( $m/z$ ): 362.98 ( $[\text{M}+\text{H}^+]$ , 100%), calc. 362.2.

**536-D-Lys-Ac:** ESI-MS ( $m/z$ ): 362.98 ( $[\text{M}+\text{H}^+]$ , 100%), calc. 362.2.

**539-D-Lys-Ac:**  $^1\text{H-NMR}$  (400 MHz, MeOD)  $\delta$  6.95-6.86 (m, 3H), 4.38-4.35 (m, 1H), 3.18 (t,  $J = 6.99$  Hz, 2H), 2.75-2.71 (m, 4H), 2.55 (t,  $J = 7.76$ , 2H), 2.18 (t,  $J = 7.75$  Hz, 2H), 1.98 (s, 3H), 1.92-1.83 (m, 3H), 1.81-1.78 (m, 4H), 1.75-1.66 (m, 1H), 1.58-1.38 (m, 4H). ESI-MS (m/z): 389.01 ( $[\text{M}+\text{H}^+]$ , 100%), calc. 388.24.

**624-D-Lys-Ac:**  $^1\text{H-NMR}$  (400 MHz, MeOD)  $\delta$  7.43 (d,  $J = 8.37$  Hz, 2H), 7.14 (d,  $J = 8.37$  Hz, 2H), 4.38-4.34 (m, 1H), 3.18 (t,  $J = 6.91$  Hz, 2H), 2.61 (t,  $J = 7.61$  Hz, 2H), 2.26 (t,  $J = 7.61$  Hz, 2H), 1.99 (s, 3H), 1.95-1.83 (m, 3H), 1.76-1.66 (m, 1H), 1.58-1.38 (m, 4H). ESI-MS (m/z): 412.85 / 414.85 ( $[\text{M}+\text{H}^+]$ , 100%, 98%), calc. 412.1 / 414.1.

**428-D-Lys-FAM:**  $^1\text{H-NMR}$  (400 MHz,  $\text{d}_6\text{-DMSO}$ )  $\delta$  8.97 (d,  $J = 8.14$  Hz, 1H), 8.57 (s, 1H), 8.28 (d,  $J = 8.14$  Hz, 1H), 7.82-7.8 (t,  $J = 5.56$  Hz, 1H), 7.58 (d,  $J = 8.1$  Hz, 2H), 7.27 (d,  $J = 8.1$  Hz, 1H), 6.96 (d,  $J = 8.24$  Hz, 2H), 6.69 (d,  $J = 2.15$  Hz, 2H), 6.6-6.52 (m, 4H), 4.42-4.38 (m, 1H), 3.06-3.02 (m, 2H), 2.46 (t,  $J = 7.38$  Hz, 2H), 2.02 (t,  $J = 7.38$  Hz, 2H), 1.88-1.70 (m, 4H), 1.45-1.34 (m, 4). ESI-MS (m/z): 776.95 ( $[\text{M}+\text{H}^+]$ , 100%), calc. 776.12.

**428-D-Lys- $\beta$ Ala-DTPA-Gd:** ESI-MS (m/z): 1185.06 ( $[\text{M}+\text{H}^+]$ , 100%), calc. 1184.17

In addition to NMR and MS all compounds were characterized by HPLC/UV (Supplementary Figure 5).

### HPLC

All reaction products were purified by HPLC on a Waters XTerra Prep RP<sub>18</sub> column (5  $\mu\text{M}$ , 10 x 150 mm) using a linear gradient from 0.1% trifluoroacetic acid to acetonitrile or from 100 mM TEAA, pH 7 to 100 mM TEAA in 80% acetonitrile, pH 7 in 15 minutes. Unless one gradient is specifically indicated both could be used for purification. After collection of the desired fractions, solvents and buffer were removed under vacuum.

### Isothermal Titration Calorimetry

Isothermal titration calorimetry measurements were performed using a VP-ITC instrument (Microcal). Fatty acid free HSA (Sigma, A1887) or fatty acid free MSA (Sigma, A1056) was dissolved in PBS, 2% DMSO and dialyzed against the identical buffer. The concentration of protein for the experiment was varying between 30  $\mu$ M to 1 mM. HSA or MSA was titrated with a solution of binding molecule roughly ten fold higher in concentration than the protein solution in PBS, 2% DMSO at 37 °C. Typically, titrations were performed until a ratio of 2 : 1 of ligand to binding sites on protein was reached. The ligand had been dissolved in the dialysis buffer. The concentration of binding molecule was determined by HPLC-UV at 260 nm allowing precise measurements without any contribution of the solvent. The corresponding spectral information is given in Supplementary Figure 5-7. The resulting titration curves were processed and fitted with the Origin 7 software (Microcal) to obtain  $K_d$  and  $\Delta H$  values. The affinity of **326-D-Lys-Ac** could not be determined due to insolubility of the conjugate.

### Band-shift Assay

Fluorescein, phenethylamine-FAM, **428-D-Lys-FAM**, and **622-D-Lys-FITC** at a concentration of 5  $\mu$ M were incubated with PBS, HSA (50  $\mu$ M) and human serum (10x diluted in PBS) in PBS. After one hour the samples were loaded onto a 20% polyacrylamide gel and run for 40 minutes at 180 V in Tris/Borate/EDTA pH 8.2.

### Fluorescence Polarization

100 nM of fluorescein, **428-D-Lys-FAM**, **622-D-Lys-FAM** and phenethylamine-FAM were incubated with increasing amounts of HSA in PBS for 1 h at 25 °C. The fluorescence polarization was determined by excitation at 485 nm and measurement at 535 nm.

## Competition Experiments

500 nM of **428**-D-Lys-FAM and HSA were incubated with 1  $\mu$ M, 10  $\mu$ M and 100  $\mu$ M of competitor in PBS for 1 h at 25 °C. The fluorescence polarization was determined by excitation at 485 nm and measurement at 535 nm.

## Pharmacokinetics of fluorescein derivatives

Pharmacokinetic analyses were performed under the Project Licenses 198/2005 of the Veterinäramt des Kantons Zürich (issued to D.N.). 129SvPas mice were injected into the tail vein with 20 nmol of fluorescein, Phenethylamine-FAM, **428**-D-Lys-FAM, or **622**-D-Lys-FAM dissolved in 100  $\mu$ l of 100 mM Tris-HCl, 105 mM NaCl, pH 8.2 (2 mice each). 20-30  $\mu$ l blood samples were taken from the vena saphena with EDTA coated capillaries (Sarstedt) 1, 5, 15, 30, 60, 120, 240, 360, 480 and 1440 minutes after injection and centrifuged. 5 - 10  $\mu$ l of plasma were mixed with 40  $\mu$ l of 0.1 % trifluoroacetic acid and 200  $\mu$ l of ethanol and incubated on ice for 1 hour. After one hour the samples were centrifuged to remove the precipitated proteins. The supernatant was dried under vacuum and subsequently redissolved in 50  $\mu$ l of DMSO / H<sub>2</sub>O. 40  $\mu$ l were injected into an LC/MS/MS system (Micromass Quattro micro API) and run on a linear gradient in 0.1% HCOOH from 5% to 95 % acetonitrile in ten minutes on a Waters XTerra MS C<sub>18</sub> column (3.5  $\mu$ M, 1 x 50 mm) while observing the daughter ions with m/z of 287.1 and 202.1 of the parent ion 333.1 for fluorescein, with m/z of 287.3 and 332.3 of the parent ion 480.2 for phenethylamine-FAM, with m/z of 287.3, 377.2 and 505.2 of the parent ion 777.1 for **428**-D-Lys-FAM, and with m/z of 287.3 and 505.4 of the parent ion 651.3 for **622**-D-Lys-FAM. Comparing the area under the curve with a dilution series of fluorescein and its derivatives in plasma followed by identical sample preparation allowed the quantification of the molecules.

### Pharmacokinetics of MSA

75 nmol MSA were labeled with DTPA by reaction with 2.1  $\mu\text{mol}$  p-SCN-Bn-DTPA in PBS for 4 h at 4 °C followed by purification on a PD-10 column (GE Healthcare). 50 nmol MSA-DTPA were labeled with 50  $\mu\text{Ci}$   $^{177}\text{Lu}$  (Perkin Elmer) and purified on a PD-10 column equilibrated in PBS. 3  $\mu\text{Ci}$  of MSA-DTPA- $^{177}\text{Lu}$  were injected into the tail vein of 129SvPas mice (2 mice). Blood sampling was performed 1, 15, 30, 60, 120, 240, 480, 1440 min after injection as described above. The amount of  $^{177}\text{Lu}$  in the injection solution and plasma was quantified by counting on a Packard Cobra  $\gamma$ -counter.

### Scanning-laser ophthalmoscopy and fluorescein angiography

Scanning-laser ophthalmoscopy imaging was performed with a Heidelberg Retina Angiograph (HRA I, Heidelberg Engineering, Germany), a confocal scanning-laser ophthalmoscope, according to previously described procedures<sup>[3]</sup>. Briefly, wild-type C57BL/6 mice were anaesthetized with ketamine (66.7 mg kg<sup>-1</sup>) and xylazine (11.7 mg kg<sup>-1</sup>), and their pupils were dilated with tropicamide eye drops (Mydriaticum Stulln, Pharma Stulln, Germany). The HRA features two argon wavelengths (488 nm and 514 nm) in the short wavelength range and two infrared diode lasers (795 nm and 830 nm) in the long wavelength range. The laser wavelength of 488 nm was used for fluorescein angiography, with a barrier filter at 500 nm. Four mice each received either 50 nmol of fluorescein or 428-D-Lys-FAM dissolved in 100  $\mu\text{l}$  of 100 mM Tris-HCl, 105 mM NaCl, pH 8.2 injected i.v. into the tail. Images were obtained at time-points 1, 5, 20 and 60 minutes after injection applying the same settings of the equipment. In particular, the brightness value in this study was fixed so that the brightest image (**428-D-Lys-FAM** at 1 minute following injection) could be recorded without overexposure. All examinations were approved by the local authorities (Anzeige v. 13.12.2006, RP Tuebingen) and were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Pharmacokinetics of DTPA complexes

Complex formation was performed by incubating 2.25 nmol of DTPA or **428-D-Lys-DTPA** with 70  $\mu\text{Ci}$  of  $^{177}\text{Lu}$  (Perkin Elmer) overnight at 25 °C in 250  $\mu\text{l}$  of PBS. Complete complex formation was verified by HPLC using a radioactivity detector (GABI Star, Raytest) with TEAA buffer. 129SvPas mice (2 mice each) were injected with 30  $\mu\text{Ci}$  of DTPA- $^{177}\text{Lu}$ , or **428-D-Lys- $\beta$ -Ala-DTPA- $^{177}\text{Lu}$**  in 100  $\mu\text{l}$  of PBS into the tail vein. Blood sampling and  $^{177}\text{Lu}$  quantification was performed 1, 5, 15, 30, 60, 120, 240, 480, 1440 min after injection as described above.

### Magnetic Resonance Imaging

All animal experiments were carried out in strict adherence to the Swiss law for animal protection. MR measurements were performed on a Bruker PharmaScan 47/16 MR system (Bruker BioSpin GmbH) operating at 200 MHz using a circular cryogenic surface coil for signal transmission and reception. Mice were anesthetized with 1.4–1.5% isoflurane in air:O<sub>2</sub> (4:1). Scout scans were performed to assess the anatomy of the animal. The shortening effect of both contrast agents DTPA-Gd and 428-D-Lys- $\beta$ -Ala-DTPA-Gd on the longitudinal blood relaxation time (T<sub>1</sub>) was investigated in two C57/Bl6 mice. For this purpose, transverse cine 2D images were acquired using the following sequence parameters: field of view (FOV): 19x19x0.5mm<sup>3</sup>, matrix size: 128x128, flip angle: 15°, TE/TR: 3.0/14ms, averages: 6, repetitions: 50, temporal resolution: 10.75s, total scan time: 8min 57s. The inflowing blood was saturated by exciting a 4mm-thick slice (flip angle: 15°) cranial to the imaging plane. Contrast agents were injected into the tail vein after a baseline of twenty repetitions. Firstly, a dose of 1.2  $\mu\text{mol}$  of DTPA-Gd and secondly, after a wait time of 30min, a dose of 1.2  $\mu\text{mol}$  of 428-D-Lys- $\beta$ -Ala-DTPA-Gd was administered. The time course of the MR signal was analyzed in a large vessel and normalized to the baseline value for comparison.

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