Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Spherical Nucleic Acid Nanoparticle Conjugates Enhance G-Quadruplex Formation and Increase Serum Protein Interactions**

*Alyssa B. Chinen, Chenxia M. Guan, and Chad A. Mirkin**

anie_201409211_sm_miscellaneous_information.pdf

MATERIALS AND METHODS

Spherical Nucleic Acid Nanoparticle Synthesis.

DNA was synthesized on an MM48 Oligonucleotide synthesizer (BioAutomation) using standard solid-phase synthesis and reagents (Glen Research). DNA was purified by HPLC on a Microsorb C18 column (Varian). Citrate-stabilized 13nm (±1nm) AuNPs were synthesized via the Frens method. 30nmole thiolated DNA was added to 10ml 10nM AuNPs and incubated for 2 hours at RT. TWEEN-20 was added to 0.2% and NaCl was added to 100mM. Following this, the NaCl concentration was gradually increased by 100mM every 30 minutes to a final concentration of 0.5M and 1M for G-rich and poly-T SNAs, respectively. AuNP and DNA concentrations were determined by measuring their absorbance at 524nm and 260nm, respectively using a Cary 5000 UV-Visible spectrophotometer (Agilent).

Quantification of SNA Oligonucleotide Loading.

To determine the number of oligonucleotides per SNA, an OliGreen (Life Technologies) assay was performed. 0.2pmol of SNAs were dissolved with 40mM KCN to release the bound oligonucleotides. The concentration of oligonucleotides was then determined by measuring the fluorescence of OliGreen dye binding to the released oligonucleotides, and comparing to a standard curve prepared using the sample sequence. To calculate the oligonucleotide loading, the total number of DNA strands released was divided by the total number of SNAs.

Incubation of SNAs with Human Serum and Isolation of Specifically Bound Proteins.

SNAs (5nM) were incubated in 10% type AB male human serum (HS) (Sigma) for 24 hours at 37°C with shaking. Unbound protein was removed by centrifugation (3X). The concentration of purified SNAs with proteins bound was determined, and adjusted to 120nM by dilution with PBS. SDS was added to the protein coated SNAs at a final concentration of 1%, and heated for 5 minutes at 95°C to release the specifically bound proteins. The mixture was then centrifuged, and the supernatant was collected to analyze the specifically bound proteins. SNAs were collected from the pellet and further analyzed.

DLS and Zeta Potential Measurements.

Dynamic light scattering (DLS) and zeta-potential measurements were taken using a Malvern Zetasizer NanoZS. Samples were prepared in PBS at a concentration of 1nM SNA and measurements were taken at 25°C. For diameter, the average and standard deviation of 5 measurements are reported, and for zeta-potential, the average and standard deviation of 3 measurements are reported.

Protein Quantification.

The BCA assay was used to quantify protein bound to SNAs following incubation in human serum. Protein samples were isolated from 1pmol SNA following the procedure described previously, and were analyzed using the BCA Protein Assay Kit (Pierce), and using bovine serum albumin to generate a standard curve from 0-250ug protein.

SDS-PAGE.

Proteins harvested from 3pmol SNA were analyzed by SDS-PAGE using pre-cast Mini ProTEAN TGX 4-15% polyacrylamide gels (BioRad). To compare the amount of protein from each type of SNA, the protein samples loaded were isolated from the same vol-

ume and concentration of SNAs. Spectra MultiColor Broad Range Protein Ladder was used as a molecular weight marker (Pierce) and the gels were run for 1 hour at 100V in Tris-Glycine-SDS (TGS) buffer. Gels were then stained using IR Blue Protein stain and imaged on an Odyssey imager at 700nm (LI-COR Biosciences).

Protein digestion, mass spectrometry of peptides, and protein identification.

Protein samples isolated as previously described were prepared for mass spectrometry analysis by first acetone precipitating to remove SDS. The samples were denatured in 8M urea, and reduced using DTT and alkylated using iodoacetamide. The urea was then diluted to 1M and protein was processed by proteolytic digestion with sequencing grade trypsin at 37°C overnight. The samples were desalted and loaded into a 10cm long, 75μM reverse phase capillary column (ProteoPep II C18, 300Å, 5μm size, New Objective) and separated using a 100min gradient from 5-100% acetonitrile on a Proxeon Easy n-LC II (Thermo Scientific). Next, the peptides were eluted into an LTQ Orbitrap Velos mass spectrometer (Thermo Scientific) with electrospray ionization at 350nL/min flow rate. The mass spectrometer was run in a data-dependent mode, where 10 of the most intense ions from each MS1 precursor ion scan were selected from fragmentation by CID (collision-induced dissociation). The parameters for mass spectrometry were as follows: the resolution of MS1 was set at 60,000, normalized collision energy was set to 35%, activation time was 10ms, isolate width was 1.5, and the +1 and +4 and higher charge states were rejected.

The results were processed using Proteome Discoverer (Thermo Scientific) and searched using an in-house MASCOT server. The results were also searched against the Swiss-Prot database, with the following database search filters: *Homo sapiens*, trypsin for enzyme specificity, cysteine carbamidomethylation for fixed modification, methionine oxidation and N-terminal acetylation for variable modification, precursor mass tolerance of ±10ppm, and a fragment ion mass tolerance of ±0.8Da. All spectra were searched against target/decoy databases, and the mascot significance threshold was chosen to achieve a targeted false discovery rate of 1%. The peptide identification was considered valid if its corresponding mascot score was equal or less than the threshold. Protein grouping was enabled in Proteome Discoverer, and were grouped to satisfy the rule of parsimony. In addition, proteins with less than three unique peptides were not considered to eliminate false discovery.

Western Blot Analysis of Proteins Bound to SNAs.

Proteins were isolated from 3pmol of SNAs as previously described. The proteins were separated using a pre-cast Mini ProTEAN TGX 4-20% polyacrylamide gel (BioRad) and were then transferred onto a nitrocellulose membrane (Thermo Scientific) using a semi-dry transfer cell (BioRad). Membranes were blocked using Odyssey blocking buffer (LI-COR Biosciences). Apolipoprotein B100 was probed using a primary goat antibody (1:1000, Abcam ab98132); factor H using a primary goat antibody (1:2000, Abcam ab36134); transferrin using a primary rabbit antibody (1:1000, Abcam ab1223); complement C3 using a primary rabbit antibody (1:500, Abcam ab97462); and serum albumin using primary goat antibody (1:1000, Abcam ab19194). The bands were labeled with the following secondary antibodies: anti-goat (1:10,000) IgG IRDye 800 (LI-COR Biosciences) and anti-rabbit (1:10,000) IgG IRDye 680 (LI-COR Biosciences). The protein bands were visualized using the Odyssey Clx Infrared Imaging System (LI-COR Biosciences).

Circular Dichroism Measurements of Free DNA and SNA Nanoparticle Conjugates.
Free DNA and SNAs were prepared in either PBS or 150mM KCl, NaCl, or LiCl at a concentration of 7.5uM and 50nM, respectively to normalize the measurement by oligonucleotide concentration. Samples were measured using a JASCO J-815 CD Spectrometer. Scans were taken from 230-300 nm with a 2nm bandwidth and a scanning speed of 100nm/minute. The data integration time was set to 2 seconds and measurements were made every 0.025nm. The spectra shown are the results of averaging 20 runs. To determine the melting temperature of G-quadruplexes, the ellipticity of G-rich SNAs (50 nM) and G-rich DNA (7.5 uM) at 260nm was measured from 20-90°C, at 1°C intervals with a data integration time of 32 seconds.

Modified ELISA Assay.
To analyze the binding affinity of G-rich and poly-T SNAs and DNA for complement factor H, a modified ELISA assay was used, following a previously published procedure.[1] Specifically, 100ul of 10ug/ml Factor H capture antibody in 50mM sodium carbonate, pH=9.5 was added per well of 96 well plate High Bind ELISA plates (Corning) and incubated overnight at 4°C while shaking. Each well was rinsed three times with 100ul PBS containing 0.05% TWEEN-20 (PBS-T). The plates were then blocked with 0.01mg/ml BSA in PBS for two hours at room temperature while shaking. The plates were rinsed again three times with 100ul PBS-T. 100ul of 5ug/ml factor H protein in 20mM Tris, 140mM NaCl, 10% glycerol and 2mM EDTA (Sigma) was then added to half of the wells. The other half served as control wells which were not coated with factor H protein, but were treated with the same coating buffer to account for any non-specific binding. The plates were incubated with factor H protein for two hours at room temperature with shaking. The plates were washed again three times with 100ul PBS-T. Samples were added to the wells (one set of wells coated with factor H protein and one set of control wells that were coated with capture antibody but not factor H). For SNAs, samples were added from 0-10nM in PBS with 0.05% TWEEN-20 and for linear DNA, samples were added from 0-1.5uM in PBS with 0.05% TWEEN-20. Samples were incubated with the coated plates for two hours at room temperature. To remove unbound oligonucleotides, the wells were washed three times with PBS-T. To release the oligonucleotides and dissolve the AuNPs, 100ul 40mM KCN in PBS with 0.1% TWEEN-20 was added to each well and heated to 95°C for 5 minutes. Oligonucloetide concentration was then calculated using OliGreen (Life Technologies) by comparison to standard curves of G and T DNA containing 40mM KCN in PBS with 0.1% TWEEN-20. The values reported are the average and standard deviation of three independent measurements.

Macrophage Uptake.
RAW 264.7 cells were cultured in DMEM media supplemented with 10% FBS and 1% penicillin/streptomycin. For uptake studies, 100,000 cells were seeded per well of a 12-well plate. The next day, cells were incubated with 1nM SNA in OptiMEM for 15 minutes, 30 minutes, or 1 hour. Cells were then washed three times with OptiMEM to remove excess SNAs. Cells were collected by scraping in DMEM and counted using a Countess Automated Cell Counter (Life Technologies). Cells were then digested in 3% HCl in HNO_3 for 1 hour at 55°C. These samples were diluted with ICP matrix containing 2% HCl and 2% HNO_3, and internal standard was added to a final concentration of 1ppb indium, bismuth, and homium. Samples were then analyzed using an X Series

II ICP-MS (ThermoFisher) to determine the Au-197 content. Reported values represent the average and standard deviation from three wells.

RESULTS

SNA Characterization

Table S1. Characterization of G-rich and poly-T SNAs

	Diameter (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)	DNA Strands/AuNP
G-rich SNAs	54±8	0.39±0.02	-21.1±0.6	134±17
Poly-T SNAs	56±3	0.42±0.34	-23.2±0.8	132±18

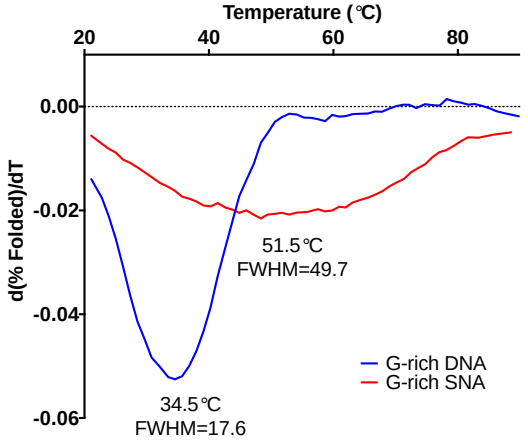

Figure S1. First derivative plot of % G-qudruplex folded as measured by CD spectroscopy. The change in ellipticity at 260nm was monitored at 20-90°C to determine the melting temperature for G-quadruplexes formed with linear G-rich DNA and G-rich SNAs. The full width half maximum of the derivative of this change signifies the cooperativity of G-quadruplex formation.

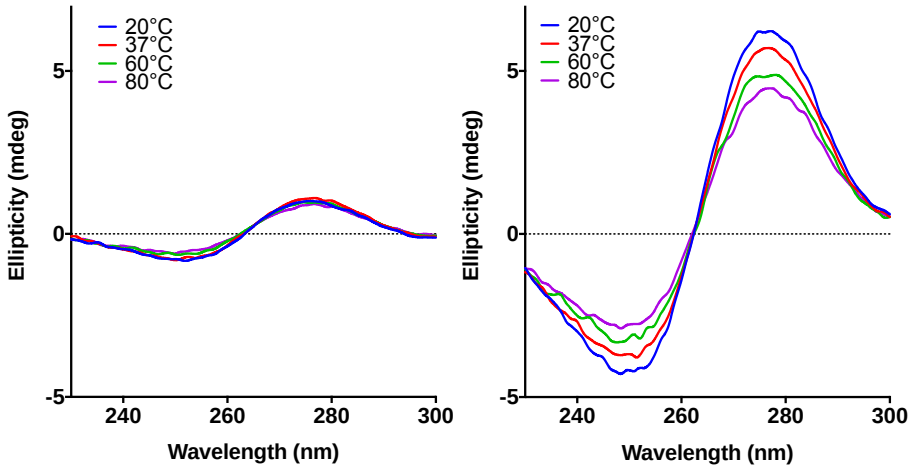

Figure S2. T_{40} DNA (left) and Poly-T SNAs (right) monitored by CD spectroscopy from 20-90°C.

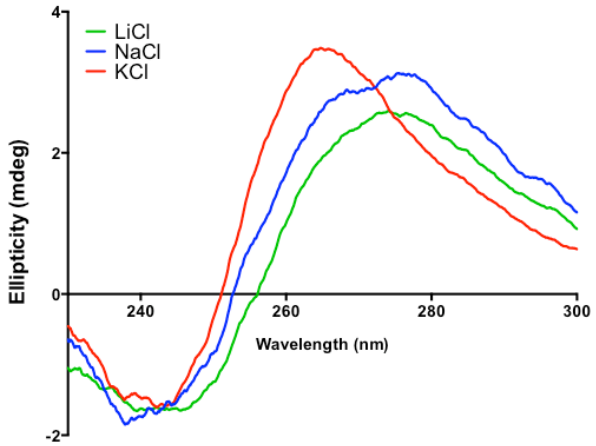

Figure S3. CD spectroscopy measuring the ellipticity of G-quadruplexes on G-rich SNAs in 150mM monovalent salt solutions (KCl, NaCl, and LiCl). It is known that KCl stabilizes parallel G-quadruplex formation, while NaCl and LiCl do so to a lesser extent.[2] Perturbation of the characteristic CD peak at 265nm in KCl in either NaCl or LiCl suggests that parallel G-quadruplexes are forming on SNAs.

Table S2. Percentage of DNA oligonucleotides remaining after serum proteins were removed from SNAs.

	% oligonucleotides remaining
G-rich SNAs	109±5
Poly-T SNAs	101±2

The percentage of DNA oligonucleotides remaining on G-rich and poly-T SNAs following incubation in 10% HS for 24 hours at 37°C and subsequent removal of the adsorbed proteins was determined using the OliGreen assay. The results confirmed that oligonucleotides remain bound to SNAs following protein adsorption and after our treatment conditions to remove bound proteins.

Table S3. Mass spectrometry identification of proteins that bound to G-rich SNAs.

Accession	Description	Coverage	# Unique Peptides	# Peptides	# PSMs	MW [kDa]
P04114	Apolipoprotein B-100	35.96	130	130	683	515.3
P01024	Complement C3	49.49	71	71	559	187.0
P02768	Serum albumin	77.67	53	53	866	69.3
P02787	Serotransferrin	48.57	34	34	259	77.0
P08603	Complement factor H	36.56	33	36	409	139.0
P03952	Plasma kallikrein	41.54	23	23	209	71.3
P02790	Hemopexin	48.48	21	21	166	51.6
P01031	Complement C5	15.93	21	21	82	188.2
B4E1Z4	Complement factor B	22.12	20	23	135	140.9
P01042	Kininogen-1	26.09	19	19	151	71.9
P06727	Apolipoprotein A-IV	50.25	19	19	108	45.4
P02647	Apolipoprotein A-I	64.79	18	18	247	30.8
P07225	Vitamin K-dependent protein S	29.88	18	18	116	75.1

Accession	Description	Coverage	# Unique Peptides	# Peptides	# PSMs	MW [kDa]
Q14520	Hyaluronan-binding protein 2	29.11	17	17	183	62.6
P02649	Apolipoprotein E	59.94	16	16	188	36.1
E9PGP2	Coagulation factor XI	32.46	16	16	114	64.0
P13645	Keratin, type I	38.87	16	18	102	58.8
P02749	Beta-2-glycoprotein 1	48.41	15	15	183	38.3
P01871	Ig mu chain C region	36.73	14	14	131	49.3
P00734	Prothrombin	25.08	14	14	98	70.0
B4E1H2	Plasma protease C1 inhibitor	37.50	14	14	82	49.7
P08519	Apolipoprotein(a)	38.30	14	14	64	501.0
P01008	Antithrombin-III	32.54	13	13	64	52.6
P04004	Vitronectin	25.94	12	12	267	54.3
P10909	Clusterin	24.05	12	12	117	52.5
P04264	Keratin, type II cytoskeletal 1	29.97	12	15	96	66.0
P00747	Plasminogen	22.96	12	12	56	90.5
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	26.61	12	12	35	66.0
Q9BXR6	Complement factor H-related protein 5	28.12	11	12	102	64.4
P02760	Protein AMBP	31.25	11	11	86	39.0
P29622	Kallistatin	25.76	10	10	50	48.5
P35527	Keratin, type I cytoskeletal 9	25.04	9	10	45	62.0
Q96PD5	N-acetylmuramoyl-L-alanine amidase	21.70	9	9	36	62.2
P01009	Alpha-1-antitrypsin	23.68	9	9	33	46.7
P36955	Pigment epithelium-derived factor	30.86	9	9	31	46.3
P02652	Apolipoprotein A-II	69.00	8	8	66	11.2
O14791	Apolipoprotein L1	29.40	8	8	66	43.9
P35908	Keratin, type II cytoskeletal 2 epi-dermal	23.94	8	13	61	65.4
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	8.92	8	8	40	103.3
F5H1A8	Gelsolin	16.10	8	8	27	81.4
P00736	Complement C1r subcomponent	12.48	8	8	19	80.1
P04003	C4b-binding protein alpha chain	14.74	7	7	42	67.0
P04196	Histidine-rich glycoprotein	15.81	7	7	27	59.5
P09871	Complement C1s subcomponent	14.68	7	7	16	76.6
P01834	Ig kappa chain C region	80.19	6	6	101	11.6
P05546	Heparin cofactor 2	10.22	6	6	28	57.0
P02743	Serum amyloid P-component	24.66	6	6	21	25.4
P01023	Alpha-2-macroglobulin	5.56	6	6	18	163.2
P01876	Ig alpha-1 chain C region	42.78	5	10	113	37.6
P01857	Ig gamma-1 chain C region	36.67	5	9	68	36.1
P00738	Haptoglobin	37.44	5	13	38	45.2
P07357	Complement component C8 alpha chain	11.82	5	5	22	65.1
P35542	Serum amyloid A-4 protein	40.77	5	5	22	14.7

Accession	Description	Coverage	# Unique Peptides	# Peptides	# PSMs	MW [kDa]
P02765	Alpha-2-HS-glycoprotein	20.71	5	5	20	39.3
P13671	Complement component C6	7.82	5	5	17	104.7
G3XAM2	Complement factor I light chain	10.94	5	5	15	65.0
P18428	Lipopolysaccharide-binding protein	19.33	5	5	12	53.3
P12259	Coagulation factor V	2.74	5	5	11	251.5
Q5T985	Inter-alpha-trypsin inhibitor heavy chain H2	7.27	5	5	10	105.2
P02747	Complement C1q subcomponent subunit C	21.22	4	4	31	25.8
P08697	Alpha-2-antiplasmin	10.18	4	4	26	54.5
P10643	Complement component C7	9.02	4	4	17	93.5
P02655	Apolipoprotein C-II	43.56	4	4	13	11.3
Q03591	Complement factor H-related protein 1	27.27	3	8	72	37.6
P01860	Ig gamma-3 chain C region	28.38	3	8	46	41.3
D6REX5	Selenoprotein P (Fragment)	8.39	3	3	25	35.1
P19012	Keratin, type I cytoskeletal 15	9.65	3	5	20	49.2
F8VV32	Lysozyme C	30.77	3	3	15	11.5
P01880	Ig delta chain C region	12.24	3	3	12	42.2
P02774	Vitamin D-binding protein	9.28	3	3	11	52.9
E7EQ48	Proteoglycan 4	4.72	3	3	10	102.4
O00391	Sulfhydryl oxidase 1	5.62	3	3	9	82.5
F5H4W9	Serum paraoxonase/arylesterase 1	11.83	3	3	8	39.7
P01011	Alpha-1-antichymotrypsin	11.11	3	3	6	47.6
Q8TDL5	BPI fold-containing family B member 1	8.26	3	3	5	52.4
F5GY80	Complement component C8 beta chain	6.43	3	3	5	60.0

Table S4. Mass spectrometry identification of proteins that bound to poly-T SNAs.

Accession	Description	Coverage	# Unique Peptides	# Peptides	# PSMs	MW [kDa]
P04114	Apolipoprotein B-100	19.72	63	63	219	515.3
P02768	Serum albumin	77.34	53	53	1178	69.3
P01024	Complement C3	27.18	31	31	123	187.0
P08603	Complement factor H	27.54	24	25	121	139.0
P02787	Serotransferrin	35.67	22	22	130	77.0
P02647	Apolipoprotein A-I	63.30	20	20	455	30.8
P03952	Plasma kallikrein	36.36	19	19	130	71.3
P06727	Apolipoprotein A-IV	45.71	17	17	73	45.4
P07225	Vitamin K-dependent protein S	28.99	16	16	136	75.1
P01042	Kininogen-1	22.20	15	15	134	71.9
P02649	Apolipoprotein E	48.90	15	15	94	36.1

Accession	Description	Coverage	# Unique Peptides	# Peptides	# PSMs	MW [kDa]
P02749	Beta-2-glycoprotein 1	57.68	14	14	156	38.3
P10909	Clusterin	22.05	11	11	118	52.5
P02790	Hemopexin	28.79	11	11	66	51.6
P01871	Ig mu chain C region	29.87	10	10	49	49.3
P01008	Antithrombin-III	26.72	10	10	32	52.6
B4E1Z4	Complement factor B	11.37	9	9	35	140.9
P04264	Keratin, type II cytoskeletal 1	19.41	9	11	30	66.0
O14791	Apolipoprotein L1	29.40	8	8	47	43.9
Q96PD5	N-acetylmuramoyl-L-alanine amidase	21.70	8	8	30	62.2
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	21.82	8	8	28	66.0
P04004	Vitronectin	19.46	7	7	76	54.3
P02652	Apolipoprotein A-II	69.00	7	7	62	11.2
B4E1H2	Plasma protease C1 inhibitor	18.75	7	7	43	49.7
P01009	Alpha-1-antitrypsin	20.10	7	7	37	46.7
E9PGP2	Coagulation factor XI	19.02	7	7	34	64.0
E9PIT3	Thrombin light chain	16.47	7	7	29	65.4
P13645	Keratin, type I cytoskeletal 10	16.27	7	7	14	58.8
P01834	Ig kappa chain C region	80.19	6	6	93	11.6
P01857	Ig gamma-1 chain C region	38.79	6	8	73	36.1
P00738	Haptoglobin	35.47	6	11	37	45.2
P04196	Histidine-rich glycoprotein	13.90	6	6	35	59.5
D6R934	Complement C1q subcomponent subunit B	39.04	6	6	18	26.4
P01876	Ig alpha-1 chain C region	40.51	5	9	45	37.6
P04003	C4b-binding protein alpha chain	11.06	5	5	19	67.0
P02774	Vitamin D-binding protein	20.89	5	5	12	52.9
K7ERI9	Truncated apolipoprotein C-I (Fragment)	31.17	4	4	34	8.6
P02747	Complement C1q subcomponent subunit C	21.22	4	4	20	25.8
P35908	Keratin, type II cytoskeletal 2 epidermal	13.15	4	6	18	65.4
P29622	Kallistatin	11.71	4	4	17	48.5
E7EQ48	Proteoglycan 4	6.54	4	4	15	102.4
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	3.87	4	4	9	103.3
F8W6P5	LVV-hemorphin-7 (Fragment)	56.67	4	4	9	9.7
K7ER74	Apolipoprotein C-II	25.84	3	4	17	20.0
P35527	Keratin, type I cytoskeletal 9	12.04	3	3	7	62.0
P01023	Alpha-2-macroglobulin	3.26	3	3	6	163.2
O00391	Sulfhydryl oxidase 1	5.62	3	3	5	82.5
P04217	Alpha-1B-glycoprotein	7.07	3	3	5	54.2

The categories in Table S4 and S5 are as follows: accession represents the protein's Uniprot identification number; coverage describes the percentage of the database protein sequence identified by the peptides analyzed; number of peptides represents the number of different peptides used to identify the protein while the number of unique peptides represents the number of different peptides that were unique to that specific protein; the number of peptide spectral matches (PSMs) corresponds to the total number of times a peptide was analyzed using mass spectrometry; and the molecular weight (MW) of the proteins were attained from Uniprot.

REFERENCES

[1] C. H. Choi, L. Hao, S. P. Narayan, E. Auyeung, C. A. Mirkin, *Proceedings of the National Academy of Sciences of the United States of America* **2013**, *110*, 7625-7630.

[2] a) A. Wong, G. Wu, *Journal of the American Chemical Society* **2003**, *125*, 13895-13905; b) A. Ambrus, D. Chen, J. Dai, T. Bialis, R. A. Jones, D. Yang, *Nucleic acids research* **2006**, *34*, 2723-2735; c) Y. Xu, H. Sugiyama, *Nucleic acids research* **2006**, *34*, 949-954; dA. N. Lane, J. B. Chaires, R. D. Gray, J. O. Trent, *Nucleic acids research* **2008**, *36*, 5482-5515.