SUPPORTING INFORMATION

Protein Corona Significantly Reduces Active Targeting Yield

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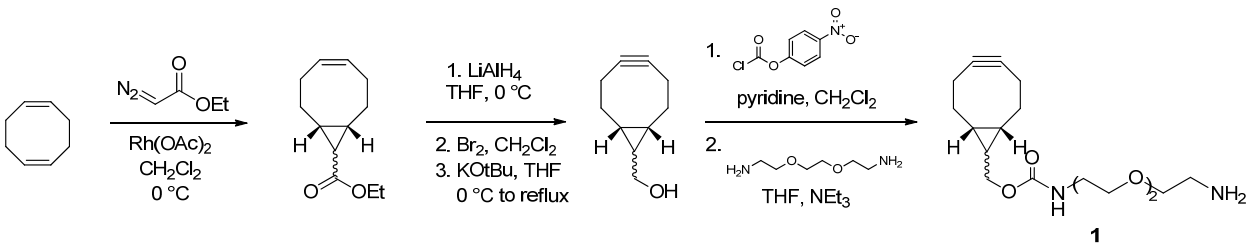
Materials and Methods

All the chemicals were purchased form Sigma-Aldrich unless otherwise noted. Silicon substrates (5 mm by 5 mm diced silicon wafers) were purchased from Ted Pella, Inc. Phosphate buffered saline (PBS) was purchased from Lonza. Fetal bovine serum (FBS) was purchased from HyClone Laboratories. Bicyclononye (BCN) **1** was synthesized as shown in Scheme S1 following previously reported procedures.[1] 11-Azidoundecyltrimethoxysilane **2** was synthesized from 11-bromoundecyltrimethoxysilane (GelSet, Inc.) as previously reported.[2] Sequencing Grade Trypsin was purchased from G-Biosciences (St. Louis, MO). Fluorescent microscopy images were acquired using a Leica CTR 6000. SEM images were acquired on a Hitachi S4800 high resolution Scanning Electron Microscope (SEM). X-ray photoelectron spectrometry (XPS) spectra were acquired on a Physical Electronics PHI 5400. Size measurements were acquired using a ZetaPlus dynamic light-scattering (DLS) detector (Brookhaven Instruments). Zeta potential was measured with a Malvern Zetasizer 3000 (Malvern Instruments).

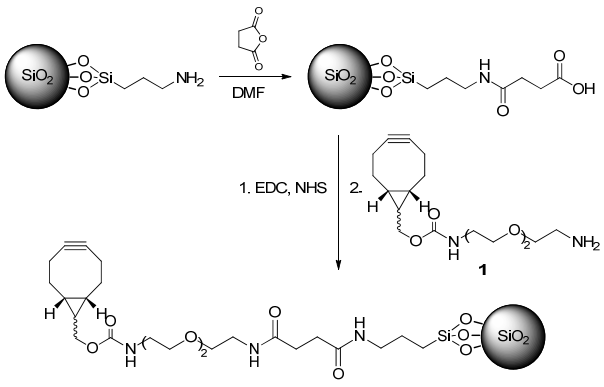


Scheme S1. Synthesis of bicyclononyne (BCN).[1]

Synthesis of fluorescent silica NPs. Fluorescent silica NPs were prepared as previously reported.[3] Briefly, 3-aminopropyltrimethoxysilane (30 mg, 0.173 mmol) was added to Rhodamine B isothiocyanate (RITC) (17 mg, 0.032 mmol) in 1 mL ethanol with triethylamine (20 µL, 0.144 mmol). The reaction was stirred for 12 h at 50 °C in the dark. The solvent was evaporated under vacuum, and the crude RITC-silane was dissolved in methanol (10 mg/mL)

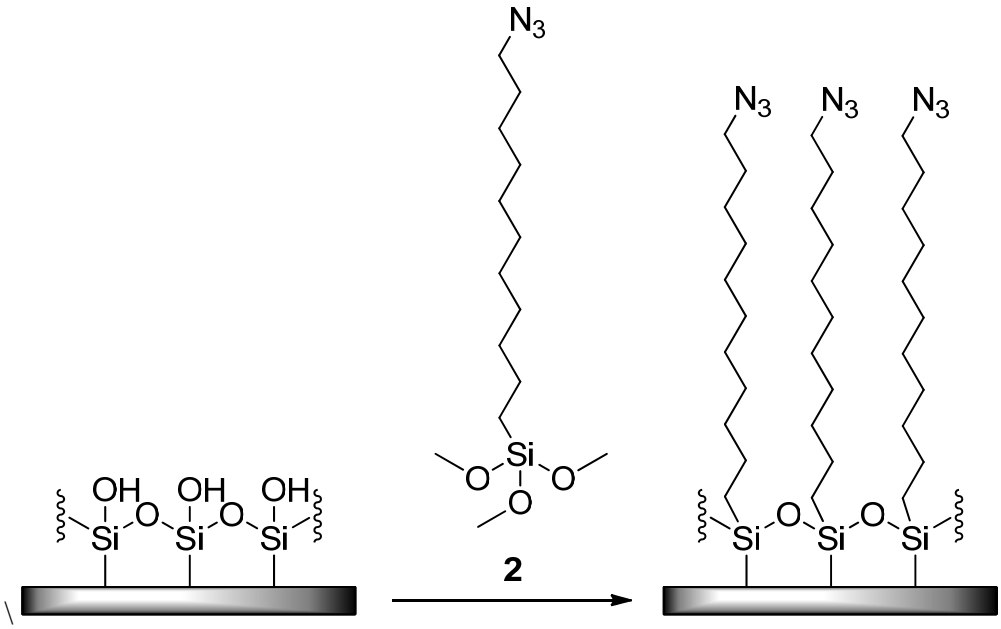
and used without further purification. Tetraethyl orthosilicate (TEOS) (62.5 μL) was added to a mixture of methanol (1 mL), DI water (0.36 mL) and concentrated ammonia (0.08 mL). The mixture was stirred at 150 rpm for 2 h, RITC-silane solution (25 μL of 10 mg/mL methanol) was added to the mixture, and the solution was stirred at 150 rpm at rt for 12 h. To functionalize the resulting silica NPs with amine moieties, 3-aminopropyltrimethoxysilane (3 μL) and TEOS (1 μL) were added to the NP solution and the reaction was stirred for 12 h. NPs were collected by centrifugation and washed with ethanol three times.

Synthesis of BCN-functionalized silica NPs (BCN-NPs). Scheme S2 summarizes the conjugation of BCN to the surface of the NPs. Silica NPs (11 mg) were dispersed in a 10% solution of succinic anhydride in DMF, and stirred overnight at rt. The NPs were collected by centrifugation, and washed three times with DMF. The resulting carboxylic acid-functionalized NPs (2 mg) were dispersed in MES buffer (1.5 mL, 0.1M, pH 6.8), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (50 mg, 0.261 mmol) and *N*-hydroxysuccinimide (50 mg, 0.434 mmol) were added to the solution. The mixture was stirred for 25 min at rt. The NPs were collected by centrifugation, washed with DI water, and then dispersed in PBS (1 mL, pH 7.4). BCN (5 mg) in methanol (50 μL) was added to NP solution and the reaction was stirred overnight. The resulting BCN-NPs were collected by centrifugation, washed three times with methanol, and suspended in DI water.[4]



Scheme S2. Surface modification of silica NPs with BCN.

Modification of silicon substrates with an azide-terminated self-assembled monolayer (SAM). Silicon substrates (5 mm by 5 mm) were cleaned in a freshly prepared piranha solution (H_2SO_4/H_2O_2, 4:1) for 30 min. (*Extreme caution must be used when cleaning with piranha solution. Gloves, goggles, and face shield should be used for protection.*) The substrates were thoroughly rinsed in deionized (DI) water and air dried. To functionalize the substrate with an azide-terminated SAM, the silicon substrates were immersed in a 70 °C solution of 11-azidoundecyltrimethoxysilane in toluene (1:9 v/v) with a trace amount of water (1 drop) for 2 h with occasional stirring (Scheme S3).[5] After cooling to rt, the substrates were sonicated in fresh toluene for 5 min, washed with acetone, dichloromethane, methanol and DI water, and air dried.[6] XPS analysis of the substrates confirmed the presence of the terminal azides on the SAM (Figure S1).



Scheme S3. Modification of silicon substrate with an azide-terminated SAM.

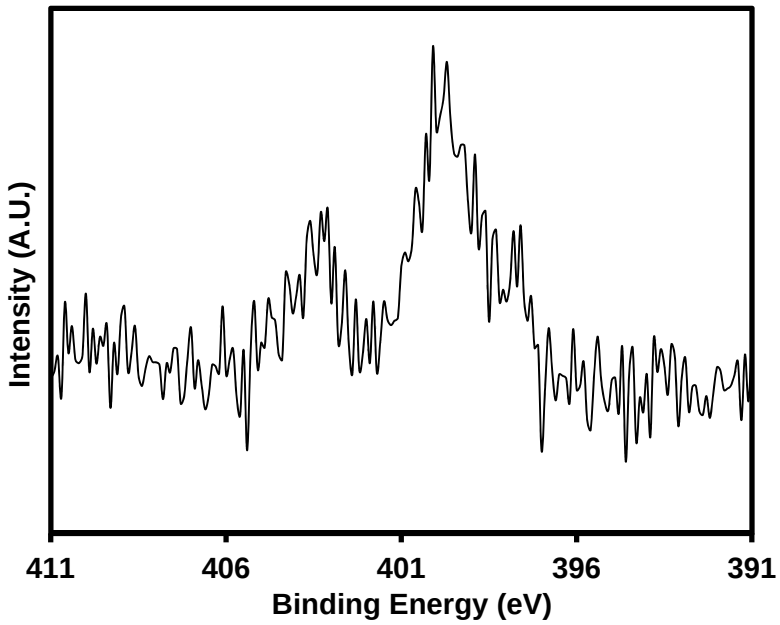


Figure S1. XPS data of SAM-modified silicon substrates confirms the presence of terminal azides. The photoelectron spectra of the nitrogen 1s binding energies shows the two peaks at 400 and 404 eV that are characteristic of azides.[7]

Size and zeta potential analysis. Measurements were made on pristine, 10% serum corona, and 100% serum corona BCN-NPs that were dispersed in PBS at a concentration of 100 μg/mL. The results are summarized in Table S1.

Table S1. Size (hydrodynamic diameter, d) and size distribution (polydispersity, PDI), determined by DLS, and zeta potential of BCN-NPs in PBS.

	pristine	10% serum corona	100% serum corona
size (d, nm)	154	383	634
polydispersity index	0.19	0.28	0.34
zeta potential (mV)	-18.9	-16.6	-7.9

Preparation of hard corona NPs. The adsorption of serum proteins to the BCN-NPs was probed by exposing the NPs to mediums containing 10% or 100% FBS. An aqueous solution of BCN-NPs (100 μL, 2 mg/mL in DI water) was mixed with 900 μL of FBS solution (10% FBS in PBS, or 100% FBS, respectively), and the solution was incubated at 37 °C for 1 h. This incubation time was selected because it is reported to produce a protein corona with a relatively stable composition, and little change in composition occurs after 1 h.[8] Each sample of BCN-NPs in the FBS solution was centrifuged at 10,000g for 30 min, the supernatant was carefully removed, and the protein-coated BCN-NPs were suspended in 500 μL of PBS. To ensure the excess (unbound or loosely bound) proteins were removed from the BCN-NP solution, which is critical for subsequent analysis of the protein composition of the hard corona on the BCN-NPs, the centrifugation process was repeated three times, and then the BCN-NPs were dispersed in cold PBS.

Conjugation of BCN-NPs to silicon substrates. For each type of BCN-NP (pristine BCN-NPs, 10% serum corona BCN-NPs, and 100% serum corona BCN-NPs), a NP solution (200 μg of NPs in 500 μL of PBS) was added to an azide-modified silicon substrate and incubated for 90 min at rt while stirring at 300 rpm. Non-specific BCN-NP binding was assessed by incubating a solution of pristine BCN-NPs (200 μg in 500 μL PBS) in the presence of a substrate that was not functionalized with azides for 90 min at rt while stirring at 300 rpm. For all samples, the unconjugated NPs were removed by rinsing in DI water. Then the samples were dried in ambient air, and conjugation was assessed by imaging with fluorescence microscopy and SEM.

Statistical analysis of reduction in targeting efficiency. The reduction in targeting efficiency due to protein adsorption was quantified by comparing the average numbers of (i) pristine BCN-NPs, (ii) 10% serum corona BCN-NPs, and (iii) 100% serum corona BCN-NPs on the 5 x 5 mm

azide-functionalized substrates. As a negative control, the number of pristine BCN-NPs on the 5 x 5 mm azide-free substrates was also assessed. Using Image J, a threshold fluorescence intensity level that represented NPs within the mosaic of images of the substrate was set, and the number of pixels that exceeded this threshold was counted. For each experimental condition, two 5 x 5 mm substrates were analyzed, and the average number of BCN-NPs was calculated. The conjugation efficiency of the BCN-NPs exposed to serum-containing medium was calculated by dividing the average number of 10 or 100% serum corona BCN-NPs on the substrate by the average number of pristine BCN-NPs per substrate and multiplying the resulting fraction by 100. The conjugation efficiency of the 10% and 100% serum corona BCN-NPs were 5% and 1%, respectively, that of the pristine BCN-NPs. Therefore, the targeting efficiency for the 10% and 100% serum corona BCN-NPs was 94 and 99%, respectively, lower than that of the pristine BCN-NPs.

Composition analysis of protein coronas on BCN-NPs with liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). The BCN-NPs exposed to mediums containing 10% or 100% FBS were collected by centrifugation at 20,000 g for 10 min, and the pellet was resuspended in 25 of Sequencing Grade Trypsin (25 μL, 12.5 ng/μL in 25 mM ammonium bicarbonate), and digested using a CEM Discover Microwave Digestor (Mathews, NC) for 15 min at 55 °C (70W). Digestion was stopped by the addition of 200 μL of an acetonitrile/water/formic acid (50:45:5 v/v/v) solution. The solvents were removed using a Thermo SpeedVac, and the residue was dissolved in 13 μL of aqueous acetonitrile (5% acetonitrile) containing 0.1% formic acid. Samples (10 μL) were analyzed by LC-MS/MS performed on a Waters quadrupole time-of-flight mass spectrometer (Q-ToF) connected to a Waters nano Acquity UPLC. LC was performed on a Waters Atlantis C-18 column (0.03 mm

particle, 0.075 mm x 150 mm) using a flow rate of 250 nL/min. Peptides were eluted using a linear gradient of aqueous acetonitrile (0-60% acetonitrile) containing 0.1% formic acid in 240 min. The mass spectrometer was set for data dependent acquisition, and MS/MS was performed on the most abundant four peaks detected at any given time. Data were analyzed using the Waters Protein Lynx Global Server 2.2.5, Mascot (Matrix Sciences) and Blasted against NCBI NR database specific for Bos taurus. The normalized percentage of spectral counts detected by LC-MS/MS for protein k (NpSpCk) was calculated according to Equation S1.

$$\mathrm{NpSpC_k} = \left(\frac{\left({}^{\mathrm{SpC}}/_{(\mathrm{M_w})_k} \right)}{\sum_{i=1}^{n} \left({}^{\mathrm{SpC}}/_{(\mathrm{M_w})_i} \right)} \right) \times 100 \quad (1)$$

A comprehensive list of the proteins detected in the coronas on the BCN-NPs exposed to the 10% and 100% FBS solutions are listed in Table S2. The normalized fractions of proteins with specified molecular weights ranges detected in the coronas on the BCN-NPs that were exposed to 10% and 100% FBS solutions are shown in Figure S2.

Table S2. Comprehensive list of normalized percentage of each protein identified by LC-MS/MS in the hard coronas on the BCN-NPs that were incubated for 1 h in medium containing 10% and 100% FBS.

Molecular Weight (Da)	Protein Name	NpSpC	
		10% serum corona	**100% serum corona**
451,342	dynein, axonemal, heavy chain 5	0.03	0
248,286	factor V	0.32	0
91,157	plasminogen precursor	0.19	0
90,229	BR serine/threonine kinase	0	0.13
85,836	integrin beta-6 precursor	0	0.19
71,659	E3 SUMO-protein ligase PIAS1	0	0.18
68,543	alpha-fetoprotein precursor	0.31	0.41
66,088	serum albumin	0.41	1.12
53,541	vitronectin	0.30	0
48,427	kininogen I precursor	0.28	0
48,118	kininogen II precursor	0	0.62
46,075	alpha-1-antiproteinase precursor	0.98	1.73
35,847	apolipoprotein E	2.41	0
30,253	apolipoprotein A-I precursor	6.05	5.96
15,849	hemoglobin fetal subunit beta	18.54	12.34
15,044	Chain A, A Novel Allosteric Mechanism In Haemoglobin	32.25	47.28
12,544	60S ribosomal protein L35a	0.89	0
11,195	apolipoprotein A-II precursor	16.27	10.19
2,337	fetuin	20.74	19.91

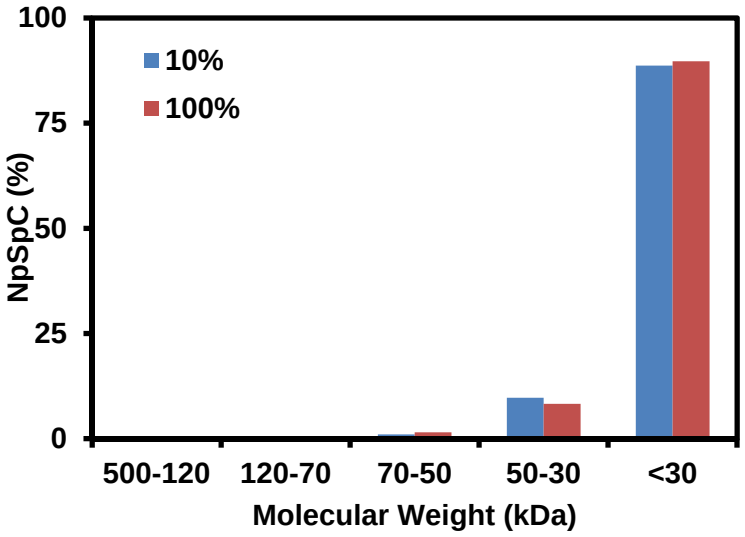


Figure S2. Normalized spectral counts (NpSpC) of proteins with the specified range of molecular weights detected in the hard coronas on the BCN-NPs incubated for 1 h in medium containing 10% and 100% FBS.

References

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