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Supporting Information

Predictive Recognition of Native Proteins by Cucurbit[7]uri in a Complex Mixture

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Table of Contents

Experimental Details

1. Materials. These commercially available materials were used without further purification: 1-amino-3 butyne, cobaltocenium hexafluorophosphate (Cob^+), ferrocenecarboxaldehyde, 11-azido-3,6,9-trioxaundecan-1-amine, sodium borohydride, methyl viologen, diethylamine (DEA), phosphate buffered saline (PBS), recombinant human insulin, bovine serum albumin (BSA), bovine carbonic anhydrase II (BCA), hen egg white lysozyme (lysozyme) and dithiothreitol (DTT), trichloroacetic acid (TCA) Sigma Aldrich HPLC protein standard mixture H2899 (Sigma Aldrich); N,N'-diethyl-1,6-diaminohexane (DEDAH) (TCI America); NHS-activated Sepharose™ 4 Fast Flow resin, Albumin & IgG Depletion SpinTrap™ (GE Healthcare); Mini-PROTEAN® TGX™ gels and 10X Tris/Glycine/SDS (Bio-Rad); SeeBlue® Plus 2 prestained standard (NOVEX Life Technologies); GelCode® Blue Stain Reagent, Pierce® Concentrator 3K MWCO, 0.5 mL (Thermo Scientific); Protein Test Mixture 6 for SDS PAGE (lyophilized powder mixture of 7 proteins, Cat. No.: 39207.01, SERVA); Human AB off-clot serum (ZenBio. Inc.); Ultrapure water with 18.2 MΩ ionic purity was used for all analytical experiments. Cucurbit[7]uril (Q7), azidobutyl–Q7 and Pericàs's catalyst were prepared as reported.[1,2,3] Recombinant human growth hormone (hGH, brand name Nutropin) was obtained from Genentech.

PBS buffer (10 mM phosphate, pH 7.4, 138 mM NaCl, 2.7 mM KCl) was prepared from commercially available, pre-packaged, dry material in ultrapure water and sterile filtered. A stock solution of 50 mM sodium acetate solution was used for the Cob^+ solutions. Protein stock solutions were prepared from dry

powder (massed to ±0.02 mg with an accuracy of at least three significant digits) and dissolved in PBS buffer. All protein solutions were stored at 4 °C.

2. Composition and Preparation of the Protein Mixtures.

Protein mixture 1 (**P1**) was composed of the seven proteins from the Serva Protein Test Mixture 6 for SDS PAGE (trypsin inhibitor from bovine lung, cytochrome C, trypsin inhibitor from soybean, BCA, albumin from egg, BSA and phosphorylase B, total protein 1.62 mg/mL) in addition to hGH (0.228 mg/mL, 10 μM), and insulin (0.5 mg/mL, 86 μM). The concentration of proteins in **P1** was estimated using densitometry as described further below.

Protein mixture 2 (**P2**) was composed of human blood serum with added hGH and insulin. The serum was thawed at 4 °C and centrifuged (5000 rpm, 1 min, room temp) before use. The supernatant was removed, stock solutions of hGH and insulin in PBS were added, and the mixture was diluted with PBS to a final concentration of 20% serum, 0.228 mg/mL (10 μM) hGH, and 0.5 mg/mL (86 μM) insulin.

3. Synthesis of the Alkyne-Resin. (Scheme S1) Commercially available NHS-activated Sepharose fast flow resin suspended in isopropanol (2 mL, ~36 μmol according to manufacturer's supplied information) was added to a 30 mL screw-capped glass vessel fitted with a coarse glass frit and stopcock (i.e., a solid-phase peptide synthesis vessel), and the isopropanol was drained completely. Separately, 1-amino-3-butyne (69 mg, 1 mmol) was dissolved in 2 mL of an aqueous solution containing 0.2 M $NaHCO_3$ and 0.5 M NaCl, pH 8.26. The

resulting alkyne solution was added to the vessel containing the freshly dried NHS-activated Sepharose resin, and the mixture was shaken for 16 h at room temp. The resulting alkyne-resin was washed with water (20 mL, 10 times) and stored as a suspension in 30 mL water at 4 °C.

4. Synthesis of the Q7-Resin. The 30 mL of stored alkyne-resin suspension described above was mixed well, 17 mL was transferred to a solid-phase peptide synthesis vessel, and the water was removed by filtration. The resin was rinsed with water (20 mL, 5 times) and drained. An aqueous solution (6 mL) containing azidobutyl-Q7 (15.86 mg, 2.08 mM) and Pericàs's catalyst[1] (1.52 mg, 0.42 mM) was added to the vessel, and the mixture was shaken at 110 rpm and 50 °C for 48 h. The solvent was drained, and the resulting Q7-resin was rinsed with water (20 mL, 10 times) and stored as an aqueous suspension in 30 mL water at 4 °C. Q7-resin should be stored in this manner for long-term stability.[4]

5. Preparing the Q7-Resin for Binding Experiments. Q7-resin was used as a dry powder for binding experiments and should be used immediately (within an hour) after drying. The dry resin was obtained as follows: a quantity of the stored suspension was filtered over a coarse glass frit, the solid resin was washed with methanol (20 mL, 3 times) and resuspended in methanol (~3 mL), and an appropriate volume of the well-mixed suspension was transferred to a pre-weighed 1.7 mL conical centrifuge tube. After centrifugation (13,200 rpm, 1 min, room temp), the methanol was decanted carefully, and the resin was dried under high vacuum in a centrifugal concentrator for 10 min at room temp. The resin mass was determined by mass difference.

6. Quantifying the Capacity of the Q7-Resin to Bind Guests (Figure S1). A standard solution of cobaltocenium (Cob^+, 15.6 μM) was prepared in 50 mM sodium acetate, and the concentration was determined by UV absorbance (ε_{261}= 34,190 $M^{-1}cm^{-1}$).[5] A standard Cob^+ stock solution (600 μL) was added to 0.46 mg of freshly dried Q7-resin in a 1.7 mL conical centrifuge tube. After mixing for 30 min at room temp, the mixture was separated by centrifugation (13,200 rpm, 4 min, room temp), and the supernatant solution was carefully decanted. The residual concentration of Cob^+ in this solution was determined by UV absorbance, and the change in concentration of Cob^+ was used to determine the quantity of Cob^+ sequestered by the Q7-resin.

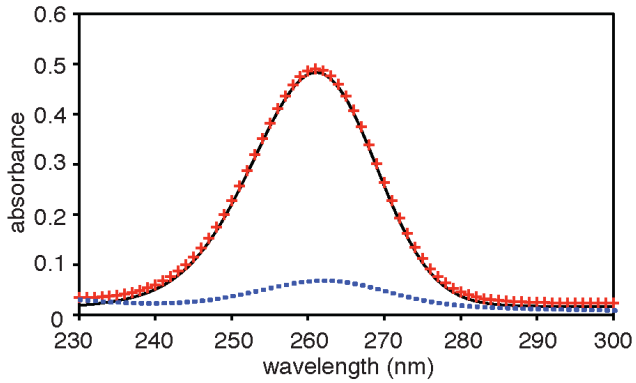

Figure S1. Representative UV spectral overlay of a 15.6 μM Cob^+ solution in 50 mM sodium acetate (black line) before and (blue ■) after treatment for 30 min with 0.46 mg Q7-resin at room temp. In this experiment, 8.0 nmol was absorbed by the Q7-resin. Treatment with alkyne-resin (red +) did not change the absorbance.

7. Control for the Covalent Attachment of Q7 to the Resin. It is possible that the experiments involving sequestering of proteins from solution by the Q7-resin could be interpreted as being due, at least in part, to Q7-azide that was not covalently attached to the resin but was, instead, nonspecifically adsorbed to the

surface of the resin, despite the extensive rinsing that follows the coupling reaction. Therefore, an additional control experiment was carried out to provide further evidence for covalent attachment of the functionalized Q7 (i.e., Q7-azide) to the Q7-resin. In this control, the coupling conditions used to attach the Q7-azide to the alkyne-resin via 1,3-dipolar cycloaddition, as described above for synthesis of the Q7-resin, were used except that the Q7-azide was replaced with regular, underivatized Q7. The assumption is that if Q7-azide will nonspecifically adsorb, so will underivatized Q7, whose structure is so similar to Q7-azide. The control reaction was conducted by mixing 7.8 mL of the 30 mL of stored alkyne resin described above with an aqueous solution (3 mL) containing 1.93 mM Q7 (underivatized) and 0.55 mg Pericàs's catalyst.[1] The mixture shook in an incubator at 110 rpm and 50 °C for 48h. The solution was drained, and the resin was rinsed with water (20 mL, 10 times) and stored as an aqueous suspension at 4 °C. A sample of this suspension was dried as described above to yield 0.77 mg powder, which was then mixed with a stock solution of cobaltocenium (Cob^+, 1 mL, 14.9 μM in 50 mM sodium acetate) for 30 min at room temp in a shaking incubator. The mixture was separated by centrifugation (13,200 rpm, 4 min), and the supernatant solution was carefully decanted. The concentration of Cob^+ in the supernatant was determined by UV absorbance using the molar absorptivity value of ε_{261}= 34,190 $M^{-1}cm^{-1}$.[5] In this case, no significant change in the concentration of Cob^+ was observed due to treatment with the resin (Figure S2). Therefore, the resin does not contain any Q7, and any Q7 that may have adsorbed onto the resin during the reaction is washed away during the rinsing

process. We assert, therefore, that the conditions used with Q7-azide, as described above for the Q7-resin synthesis, should also remove any nonspecifically adsorbed Q7-azide. Furthermore, these results show that the azide group of Q7-azide is necessary for its coupling to the alkyne resin to yield the Q7-resin, which makes sense if they are coupled covalently.

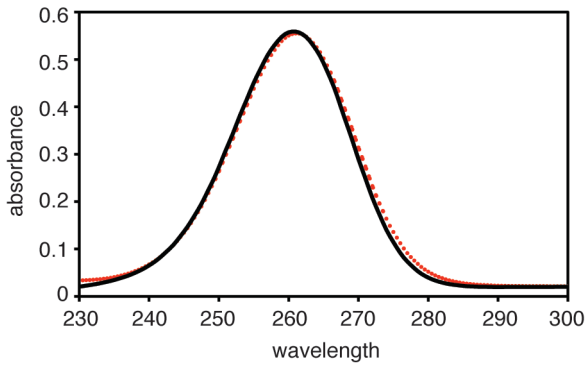

Figure S2. UV spectra at room temp of solutions containing 14.9 μM Cob^+ in 50 mM sodium acetate before (——) and after (••••) treatment with a sample of 0.77 mg resin resulting from the control experiment described above.

8. Regeneration of Q7-resin Using Q7. The Q7-resin can be regenerated after binding experiments by removal of any bound guests using a concentrated solution of underivatized Q7. A stock solution of Q7 (2 mM) in 0.1 M PBS buffer (pH 7.4) was prepared. Q7-resin collected from separation experiments was rinsed with PBS buffer (10 mL, 5 times) in a glass solid-phase peptide synthesis vessel. 10 mL of the stock Q7 solution was added to the resin, and the reaction vessel was shaken at room temp for 30 min. The solution was drained, and another 10 mL of fresh Q7 solution was added. This procedure was repeated for

a total of four Q7 rinses. The solution was then drained, and the resin was washed with PBS buffer (10 mL, 3 times) and water (20 mL, 10 times) and stored as a suspension in 30 mL water at 4 °C. The regenerated resin was quantified with Cob^+ as described above and found to have a nearly 100% recovery. For example, this process was carried out five times, and the average loading of the regenerated resin was 10.8 nmol/mg, which compares within error to the 11.0 nmol/mg loading of the freshly made Q7-resin.

9. Optimization of the Protein Separation Conditions: (1) Choice of Buffer. In the binding experiments reported by our group for cucurbit[n]urils to peptides and proteins over the past decade, we have used 10 mM sodium phosphate, pH 7.0, very consistently. Therefore, when we started these experiments, it was the first buffer we tried. We also created a simple protein mixture that would serve to differentiate a binding from non-binding protein. The protein test mixture (**TM**) was composed of BSA, BCA, hen egg white lysozyme, and human insulin. This mixture was prepared by mixing stock solutions of each protein in PBS to a final concentration of each protein at 0.232 mg/mL (molar concentrations: BSA 3.5 μM, BCA 8.0 μM, lysozyme 16 μM, and insulin 40 μM).

We were initially surprised to observe significant sequestering of *all* proteins in **TM** by both the Q7-resin and the alkyne-resin (Figure S3), and we hypothesized that this result was due to the nonspecific adsorption of proteins to the resin. We increased the ionic strength and observed a decrease in nonspecific adsorption, ultimately arriving at a choice of PBS as our buffer. PBS substantially reduced the sequestering of proteins other than insulin. This buffer

still contains 10 mM sodium phosphate at a pH near 7.0 (actually 7.4), it's salt concentration does not interfere with electrophoresis experiments, and it is commonly used in the biochemistry community. Therefore, subsequent optimization steps used PBS.

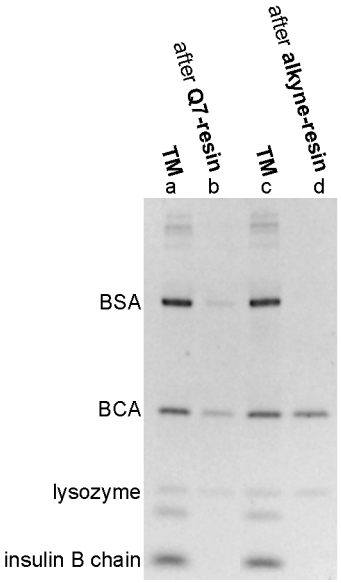

Figure S3. Optimization of buffer. SDS-PAGE gel of **TM** before (a and c) and after treatment with Q7-resin (b) or alkyne resin (d) in 10 mM sodium phosphate buffer, pH 7.0.

It is worth mentioning here that we observed other proteins in the protein mixtures interacting with the Q7-resin in PBS. These proteins, however, are not recovered after treatment with DEDAH, and thus their association with the Q7-resin is also nonspecific. As to why the proteins would nonspecifically adsorb to a greater extent with the Q7-resin than with the alkyne resin, we hypothesize that the outer surface of Q7 provides a significantly more hydrophobic surface for adsorption than that presented by corresponding alkyne groups.

Optimization of Separation Conditions: (2) Separation and Elution Times. At first, it was not clear how much time would be necessary to allow sequestering of insulin by the Q7 resin or elution of insulin from the resin by DEDAH. In our experience with solution-phase binding studies of cucurbit[n]urils, equilibrium is typical reached within the few minutes it takes to run an NMR spectrum, but in a heterogeneous system, mass transport to and from the surface of the resin becomes an issue. In order to optimize the separation time, we treated **TM** with Q7-resin for times of 1 h, 3 h, 6 h, 12 h, and 24 h in PBS (Figure S4). We observed no significant change in the amount of insulin sequestered from the **TM** mixture after 3 h. Therefore, we decided on a separation time of 3 h. In order to optimize the elution time with DEDAH, we treated **TM** with Q7-resin for 3 h and eluted with DEDAH for 1 h or 3 h (Figure S5). We observed no increase in the quantity of insulin eluted from the resin after 1 h. Therefore, we decided on an elution time of 1 h.

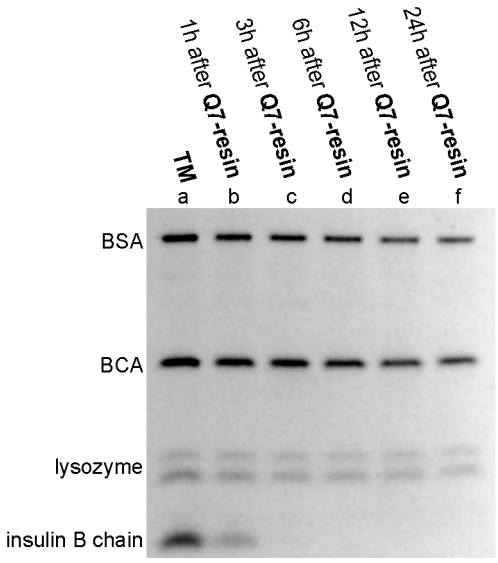

Figure S4. Optimization of separation time. SDS-PAGE gel of **TM** before (a) and after treatment with Q7-resin for times of 1-24 h (b-f).

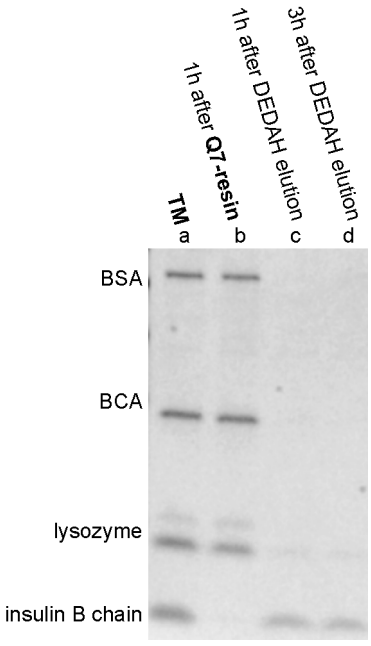

Figure S5. Optimization of elution time. SDS-PAGE gel of **TM** before (a) and after treatment with Q7-resin for 1 h (b), and then the eluted material treated with DEDAH for 1 h (c) or 3 h (d).

10. The Optimized Protein Separation Protocol. The experiments described above produced the following optimized protocol, which applies to the separation experiments described in the manuscript. A sample of protein mixture in PBS (100 μL-300 μL) was added to a sample of freshly dried Q7-resin. The separation proceeded for 3 h in a shaking incubator (110 rpm) at 37 °C. The sample was then separated by centrifugation (13,200 rpm, 3 min, room temp), and the supernatant solution was removed. A small sample (5 μL) of this supernatant was analyzed by SDS-PAGE (denoted on the gels as "Q7-resin"), as described in the following paragraph. The separated, solid Q7-resin was washed with PBS (500 μL, 3 times, 1 min each), separated by centrifugation, and resuspended in a solution containing 120 μM diethylamine (DEA, as weak eluent) in PBS (the volume of the DEA solution was the same as that of the protein mixture in the initial separation reaction), and the mixture was shaken for 1 h (110 rpm, 37 °C). The supernatant solution was then collected, and 5 μL (denoted as "DEA wash") was analyzed by SDS-PAGE. The resin was then washed with PBS (500 μL, 3 times, 1 min each) and resuspended in a PBS solution containing 4 mM N,N'-diethyl-1,6-diaminohexane (DEDAH, as strong eluent), and the elution proceeded for 1 h in a shaking incubator (110 rpm, 37 °C). For **P1**, the volume of DEDAH solution was the same as that of the protein mixture in the initial separation reaction, but for **P2**, the volume of the DEDAH solution was half of that used in the initial separation reaction. The sample was then separated by centrifugation,

and the supernatant solution was collected. A small sample (5 μL) of this solution (denoted as “DEDAH elution”) was analyzed by SDS-PAGE.

11. Control Separation Experiments Using the Alkyne-Resin. As a crucial control for the necessity of Q7 to mediate protein separation, each protein mixture was also reacted with alkyne-resin under the identical optimized protocol described above for protein separation with Q7-resin. The results of the experiments with alkyne-resin are presented next to the gels in which the protein mixtures are separated by Q7-resin.

12. Analysis of Protein Mixtures by SDS-PAGE. Each of the 5 μL samples described in the previous paragraph, as well as a 5 μL sample of the initial protein mixture, was diluted with 5 μL 1X SDS-gel loading buffer. 2 μL DTT (125 mM aqueous stock solution) was added, and the mixture was heated at 90 °C for 10 min to denature the proteins. After cooling to room temp, 8 μL of the sample was loaded onto an SDS-PAGE gel (Mini-PROTEAN® TGX™ precast gel), and electrophoresis proceeded at a constant voltage (100 V). SeeBlue® Plus 2 prestained standard (2 μL) was loaded as the molecular weight ladder. The resulting gel was stained overnight with GelCode® Blue Stain Reagent, then destained for 2 h with water, and imaged by a GelDoc™ EZ Imager (Bio-Rad). Densitometry was performed using Image Lab 4.0 (Bio-Rad).

13. Densitometry Analysis of the SDS-PAGE Data. Figures S6-S8 contain the SDS-PAGE data from Figures 2 and 3 in the main manuscript, respectively, as well as the data for the second-round of **P2** separation. The raw densitometry

data for the lanes in the gel are included at right. Table S1 lists the relative changes observed for the bands corresponding to insulin and hGH in Figure S6.

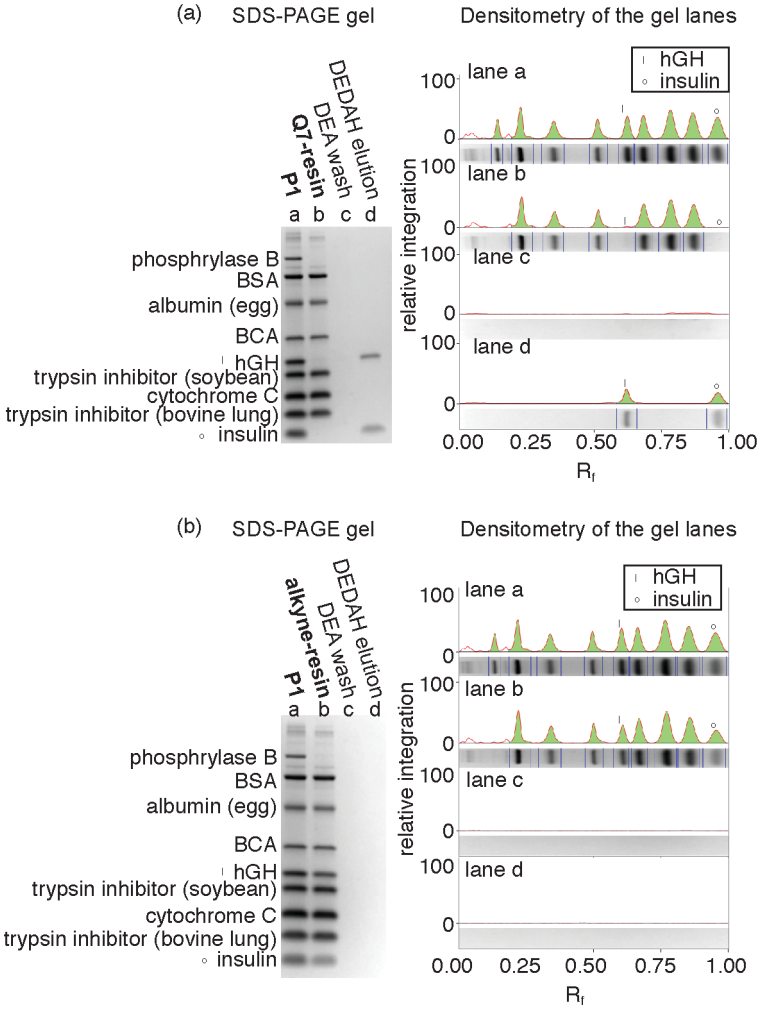

Figure S6. Representative SDS PAGE gels (left) and raw densitometry data (right) for the separation of **P1** using (a) Q7-resin, or (b) alkyne-resin. The gel data are similar to those in Figure 2 of the main manuscript. Bands for hGH and insulin are indicated on the densitometry plots.

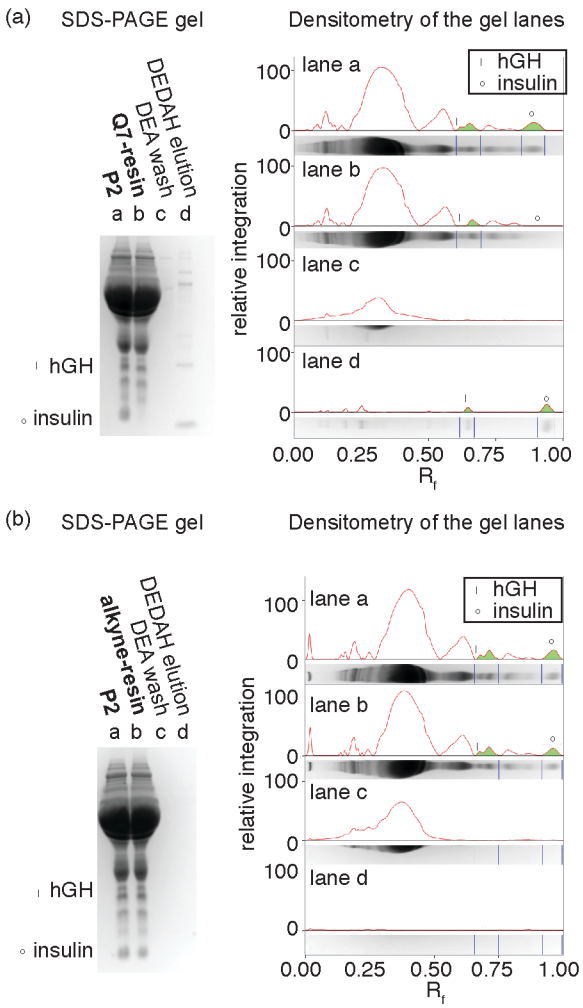

Figure S7. Representative SDS PAGE gels (left) and raw densitometry data (right) for the separation of **P2** using (a) Q7-resin, or (b) alkyne-resin. The gel data are similar to those in Figure 3 of the main manuscript. Bands for hGH and insulin are indicated on the densitometry plots.

Table S1. Quantitative results for the separation of **P1** (Figure S6).

Protein	Reduction in Intensity Due to Q7-Resin (%)[a]	Recovery by DEDAH (%)[b]	Reduction in Intensity Due to Alkyne-Resin (%)
phosphorylase B	100	0	100
BSA	6.2 (±3.9)	0	1.4
albumin (egg)	15.1 (±3.2)	0	6.6
BCA	14.7 (±1.4)	0	4.7
hGH	100	71.5 (±1.7)	25.3
soy trypsin inhibitor	22.1 (±3.1)	0	9.6
cytochrome C	5.5 (±0.7)	0	<1
bovine trypsin inhibitor	10.9 (±2.0)	0	<1
insulin	100	50.4 (±1.2)	35.7

[a] Average values from at least three experiments calculated by comparing the integrated band intensities from the Q7-resin lane to the corresponding intensities from the **P1** lane. [b] Average values from at least three experiments calculated by comparing the integrated band intensities from the DEDAH lane to the corresponding intensities from the **P1** lane, taking into account volume changes during the process.

14. Estimating the Protein Concentrations in P1. The protein concentrations in **P1** were estimated on the basis of the standard concentrations of insulin and hGH using the densitometry data and protein molecular masses (Table S2). The intensity of a band is proportional to the mass of protein, and thus the band intensity per molar mass of a protein is linearly related to its molar concentration according to the following equations:

$$\frac{intensity}{MW} = k \bullet concentration$$

$$k = \frac{\frac{intensity_{hGH}}{MW_{hGH}} - \frac{intensity_{insulin}}{MW_{insulin}}}{[hGH] - [insulin]}$$

in which the constant *k* is equal to the slope of the calibration curve, and the known values of the concentrations of hGH (10 uM) and insulin (86 uM) were used as standards.

Table S2. Estimation of the protein concentrations in **P1**.

Protein	Concentration (μM)	Concentration (mg/mL)
phosphorylase B	1.2	0.11
BSA	3.8	0.25
albumin (egg)	4.3	0.19
BCA	5.8	0.16
hGH	10	0.23
trypsin inhibitor (soy)	11	0.23
cytochrome C	27	0.33
trypsin inhibitor (bovine)	49	0.31
insulin	86	0.50

15. Fluorescence Spectroscopy. Fluorescence emission spectra were obtained at room temp with a PTI QM-4 spectrofluorometer equipped with a Xe arc lamp and digital photomultiplier, exciting at 485 nm and scanning emission from 492 to 650 nm with a step size of 5 nm. Relative fluorescence intensities (I) were determined by averaging intensity values over the range 492-650 nm.

16. Determination of the Binding Constant of Q7 with hGH. The equilibrium dissociation constant (K_d) value for the binding of hGH with Q7 was determined by competitive fluorescence titration in PBS using acridine orange (AO) as competitor and indicator. The K_d value for AO binding to Q7 was first determined in PBS by titrating a constant 5 μM AO with 0-30 μM Q7 (Figure S9) and fitting the intensity data (Figure S10) to a binary (1+1) equilibrium model to derive an average K_d value of 3.45 (± 0.02) μM, which is similar to reported values.[6,7]

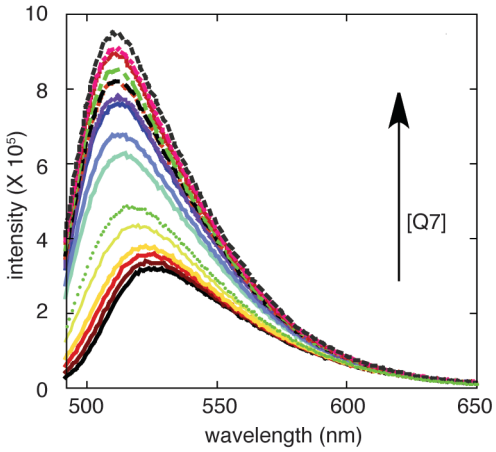

Figure S8. Fluorescence spectral overlay of AO (5 μM) in the presence of increasing concentrations of Q7 (0-30 μM) in PBS solution at room temp. Excitation was at 485 nm.

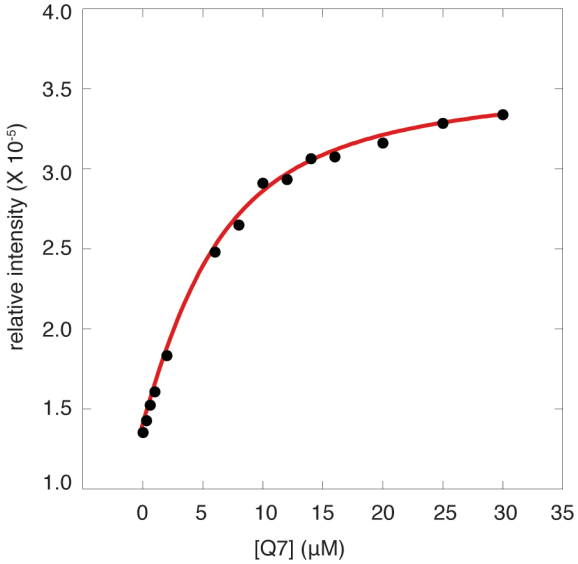

Figure S9. Representative titration curve of the data in Figure S9 fit to a binary equilibrium model.

The K_d value for the binding of Q7 to hGH was determined by competitive titration in the presence of AO. hGH competes for Q7 and displaces AO, resulting in a decrease in fluorescence intensity with increasing concentration of hGH. Samples containing 8 μM AO, 6 μM Q7 and 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16,

18 and 20 μM hGH were prepared in PBS and equilibrated overnight in the absence of light. The pipet tips and fluroresecnce cuvette were soaked in a solution of 6 μM Q7 and 8 μM AO in PBS overnight due to the adsorption of AO to the surfaces of the pipet tips and cuvette. Emission spectra of these samples were acquired (Figure S11), and the dissociation constant of hGH with Q7 was determined as 1.35 (± 0.02) μM by fitting the intensity data (Figure S11) to a competitive binding model, as described previously.[8]

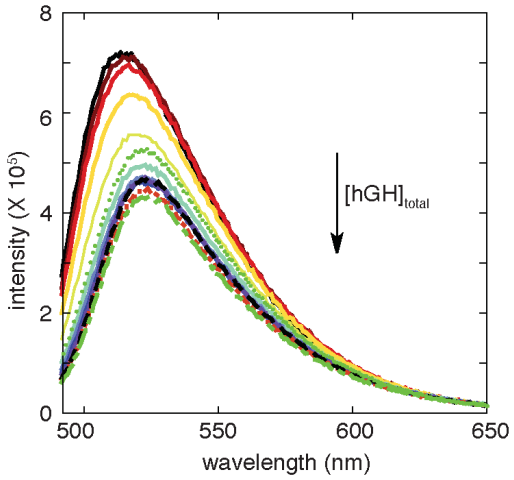

Figure S10. Fluorescence spectral overlay of AO (8 μM) in the presence of Q7 (6 μM) and increasing concentrations of hGH (0-20 μM) in PBS at room temp. Excitation was at 485 nm.

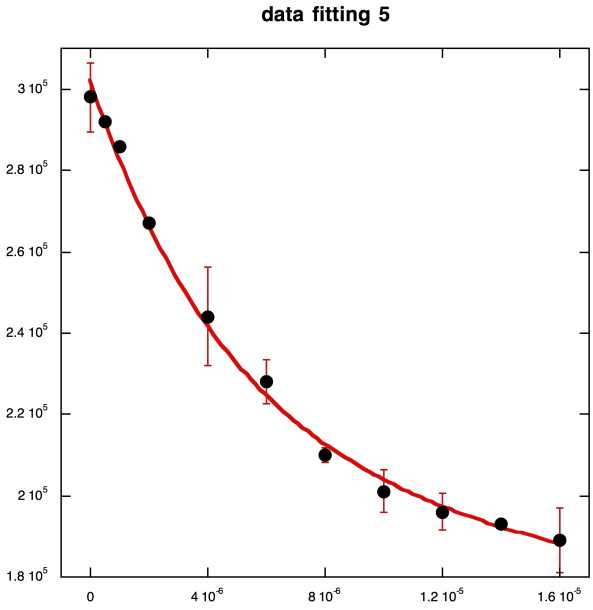

Figure S11. Titration curve of the data in Figure S11 fit to a competitive binding model.

17. Proteomic analysis of the Proteins Isolated from Serum. The separation experiment with **P2** revealed the isolation of proteins other than insulin and hGH using the Q7-resin but not the alkyne-resin. This section describes the analysis of these proteins. First, the separation experiment using Q7-resin and human serum was repeated but without adding insulin and hGH. That experiment, shown below in Figure S13, reveals the same proteins of high molecular mass that appear in Figure 3 of the main manuscript. This experiment serves to demonstrate the isolation of proteins from unmodified serum mediated by the Q7-resin but not the alkyne-resin.

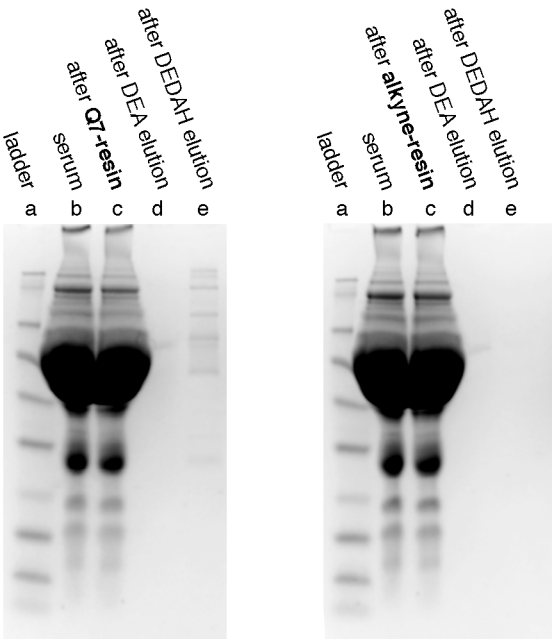

Figure S12. Representative SDS-PAGE gels of serum (diluted 5-fold with PBS) before (lane b) and after (lane c) treatment with (left) Q7-resin and (right) alkyne-resin for 3 h at 37 ^{O}C. Treatment of the resulting resin with DEA in PBS followed by DEDAH in PBS, each for 1 h at 37 ^{O}C, provided the samples shown in lane d and lane e, respectively.

Analysis of the proteins isolated from serum by Q7-resin but not alkyne-resin proceeded in several steps: a) depletion of albumin and globulin from the serum; b) separation of proteins from the serum using the Q7-resin; c) precipitation of protein from the mixture; and d) mass spectrometry analysis.

a) Depletion of Albumin & IgG from human serum. Human serum contains an exceedingly large concentration of albumin and globulin, and we carried out a protocol to deplete the serum of these proteins before treatment with the Q7-resin. A sample of thawed serum was treated with the Albumin & IgG depletion SpinTrap^{TM} from GE Healthcare Life Sciences, using the manufacturer's protocol. Briefly, the SpinTrap^{TM} column was equilibrated with the binding buffer (20 mM sodium phosphate, 0.15 M NaCl, pH7.4). 50 µL of serum was diluted to 100 µL

with the binding buffer and added to the pre-equilibrated column. After incubation for 5 min at r.t. without mixing, the sample was spun for 30 s at 2900 rpm, and the eluant was collected. The column was washed with 100 µL binding buffer, and the eluant samples were combined. An SDS-PAGE gel (Figure S14) confirmed that most of the abundant albumin and IgG proteins were deleted from the serum.

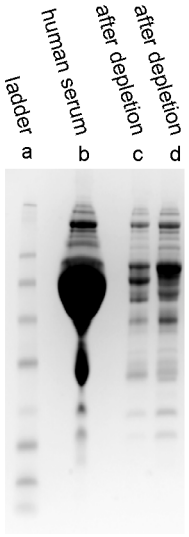

Figure S13. SDS-PAGE gel of the human serum before (lane b) and after (lanes c and d) depletion of albumin and globulin.

b) Isolation of Proteins from Human Serum using Q7-resin. 560 µL of the depleted serum was mixed with 2.38 mg Q7-resin for 3h in a shaking incubator (110 rpm) at 37 °C. The resulting resin was washed with the binding buffer (20 mM sodium phosphate, 0.15 M NaCl, pH7.4), and 112 µL of DEA (928 µM, in PBS) was added to the resin. This mixture was incubated for 1 h in a shaking incubator (110 rpm) at 37 °C. After centrifugation and removal of the supernatant, the resin was washed with PBS and then resuspended in 112 µL of

DEDAH (23 mM in PBS) and incubated for 1 h in a shaking incubator (110 rpm) at 37 °C. 5 µL of the supernatant from each step was analyzed by SDS-PAGE (Figure S15). A control experiment using alkyne-resin was also carried out.

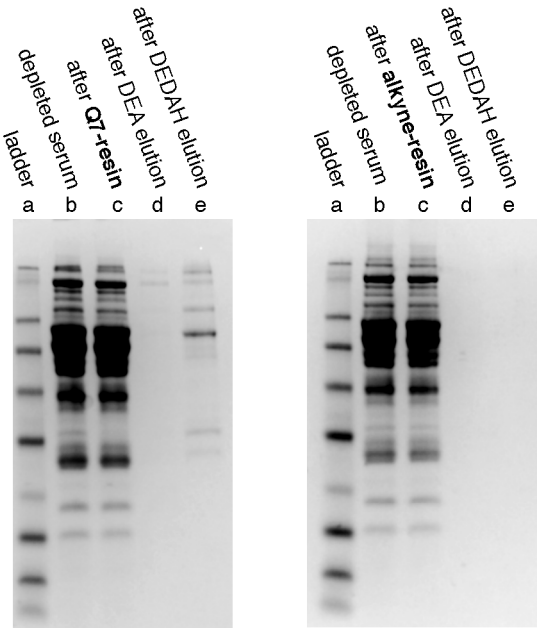

Figure S14. Representative SDS-PAGE gels of depleted serum before (lane b) and after (lane c) treatment with (left) Q7-resin and (right) alkyne-resin for 3 h at 37 ^{O}C. Treatment of the resulting resin with DEA in PBS followed by DEDAH in PBS, each for 1 h at 37 ^{O}C, provided the samples shown in lane d and lane e, respectively.

c) Precipitation of the proteins from DEDAH elution. HPLC-MS-grade acetone was stored at -80 ^{o}C and thawed from -80 ^{o}C to -20 ^{o}C just prior to use. 100% trichloroacetic acid (TCA) stock solution was prepared by dissolving 10 g TCA in water in a total volume of 10 mL and storing at 4 ^{o}C. 10% TCA solution was prepared fresh from the stock solution prior to use. Water was pre-chilled on ice and added to the DEDAH elution solution (~100 µL, see lane e in Figure S15) to a final volume of 400 µL. 100 µL of 100% TCA was added to this mixture. The sample was mixed by vortexing and incubated on ice for 15 min without shaking.

The resulting mixture was centrifuged for 20 min at 13,000 rpm at 4 ^{o}C, and the supernatant was decanted. 1 mL of 10% TCA was added to the pellet, and the mixture was mixted by vortexing and then centrifuged for 10 min at 13,000 rpm at 4 ^{o}C. After removal of the supernatant, 1 mL of cold acetone was added to the pellet. The mixture was vortexed and centrifuged for 10 min at 13,000 rpm at 4 ^{o}C. The pellet was dried under high vacuum and stored at –20 ^{o}C.

d) Mass Spectrometry Analysis. The pellets from the TCA precipitation were analyzed by the Taplin Mass Spectrometry Facility at Harvard Medical School (https://taplin.med.harvard.edu). The search results show that the sample treated with Q7-resin contains 213 possible proteins from the database, including 66 proteins with only one detected fragment. The control pellet (depleted serum treated with alkyne-resin) contains 219 possible proteins from the database, including 77 proteins with only one detected fragment. Comparing the results of the two samples, the proteins isolated by the Q7-resin but not the alkyne resin include 52 possible proteins, including 28 proteins with only one detected fragment. A list of the 24 proteins for which more than one fragment was detected is given in Table S3.

Table S3. List of Unique Proteins Isolated by Q7-Resin

Protein Number	Unique [a]	Total [b]	Reference [c,d]	Gene Symbol	Avg. Precursor Intensity ($\times 10^5$) [c]
i	24	28	VWF_HUMAN	VWF	843.0
ii	16	65	VTNC_HUMAN	VTN	315.0
iii	16	20	LG3BP_HUMAN	LGALS3BP	24.6
iv	12	16	PZP_HUMAN	PZP	14.0
v	8	11	VTDB_HUMAN	GC	15.5
vi	6	6	PAFA_HUMAN	PLA2G7	11.6
vii	5	5	TSP1_HUMAN	THBS1	6.5
viii	5	5	NID1_HUMAN	NID1	8.7
ix	4	5	FBLN1_HUMAN	FBLN1	13.9
x	4	4	A1AG2_HUMAN	ORM2	20.1
xi	4	4	FA7_HUMAN	F7	5.0
xii	3	8	PLGA_HUMAN	PLGLA	115.0
xiii	2	2	BGH3_HUMAN	TGFBI	3.8
xiv	2	2	CAH2_HUMAN	CA2	8.1
xv	2	2	CSPG4_HUMAN	CSPG4	2.5
xvi	2	2	IPSP_HUMAN	SERPINAS	4.7
xvii	1	8	HBAZ_HUMAN	HBB	5.9
xviii	1	3	KV303_HUMAN		11.9
xix	1	3	LAC7_HUMAN	IGLC7	3.4
xx	1	3	PRP1_HUMAN	PRB1	5.2
xxi	1	2	KV119_HUMAN		1.9
xxii	1	2	KV122_HUMAN		8.5
xxiii	1	2	HV103_HUMAN		10.0
xxiv	1	2	CGT_HUMAN	UGT8	17.1

[a] The number of unique peptide fragments returned from the database search algorithm for a particular protein. [b] The total number of peptide fragment matches returned from the search algorithm regardless of whether the peptide fragment is unique. [c] UniProtKB/Swiss-Prot entry name for the protein consisting of up to 11 upper case alphanumeric characters with a naming convention that can be symbolized as X_Y, where: X is a mnemonic protein identification code of at most 5 alphanumeric characters; The '_' sign serves as separator; Y is mnemonic species identification code of at most 5 alphanumeric characters. [d] Protein information can be obtained using the entry name as a search term in the RCSB Protein Data Bank. [e] The average value of the mass spectrometric intensity of all the peptide matches for each protein.

18. References

(1) Özçubukçu, S.; Ozkal, E.; Jimeno, C.; Pericàs, M. A. *Org.Lett.* **2009**, *11*, 4680.
(2) Vinciguerra, B.; Cao, L.; Cannon, J. R.; Zavalij, P. Y.; Fenselau, C.; Isaacs, L. *J. Am. Chem. Soc.* **2012**, *134*, 13133.
(3) Gao, L.; Hettiarachchi, G.; briken, V.; Isaacs, L. *Angew. Chem. Int. Ed.* **2013**, *52*, 12033.
(4) Ramalingam, V.; Kwee, S. K.; Rynoa, L. M.; Urbach, A. R. *Org. Biomol. Chem.* **2012**, *10*, 8587.
(5) Yi, S.; Kaifer, A. E. *J. Org. Chem.* **2011**, *76*, 10275.
(6) Shaikh, M.; Mohanty, J.; Singh, P. K.; Nau, W. M.; Pal, H. *Photochem. Photobiol.* **2008**, *7*, 408.
(7) Nau, W. M.; Ghale, G.; Hennig, A.; Bakirci, H.; Bailey, D. M. *J. Am. Chem. Soc.* **2009**, *131*, 11558.
(8) Logsdon, L. A.; Schardon, C. L.; Ramalingam, V.; Kwee, S. K.; Urbach, A. R. *J. Am. Chem. Soc.* **2011**, *133*, 17087.