Supporting Information for

Highly-Efficient Purification of Native Polyhistidine-tagged Proteins by Multivalent NTA-modified Magnetic Nanoparticles

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Experimental Details.

Iron oxide nanoparticle surface modification with mono-NTA Ni(II) chelate. One mL of iron oxide magnetic nanoparticles suspension (16.3 mg/mL) was added to ligand **8** (0.5 mL of a 10 mg/mL MeOH solution in 1.25 mL of distilled water). The two layers were sonicated for 1 hour, and repeatedly washed with *n*-hexanes, then methanol until the nanoparticles were readily dispersed in distilled water. Using a magnet, the nanoparticles were collected and placed in 2 mL of distilled water. The mono-NTA modified nanoparticles (0.5 mL) were placed in 1M $NiCl_2$ solution and sonicated for 1 hour. The divalent Ni(II)-loaded nanoparticles were stored in the $NiCl_2$ solution before use.

Isolation of His-Ubiquitin C-terminal Hydrolase L-1, *mouse recombinant* (6xHis-UCH-L1) with Ni(II)-loaded nanoparticles: The Ni(II)-loaded nanoparticles (10 μL; 16.3 mg/mL) were washed in distilled water (3×50 μL) and wash buffer (3×50 μL; 50 mM phosphate buffer system, 300 mM NaCl, 10 mM imidazole, pH 8.0). The washed nanoparticles were placed in 40 μL of wash buffer and 10 μL of 6xHis-UCH-L1 (1 μg 6xHis-UCH-L1/1 μL distilled water) and incubated for 60 minutes at 4 °C. Using a centrifuge and magnet, the supernatant was collected and nanoparticles washed in 50 μL aliquots (FT, W1, W2, W3). After W3, 50 μL of elution buffer (50 mM phosphate buffer system, 300 mM NaCl, 250 mM imidazole, pH 8.0) was added

and rotated for 10 minutes at 4 °C. Nanoparticles were collected in 50 μL aliquots (E1, E2). Nonspecifically bound protein was released from the nanoparticles by boiling at 95 °C for 4 minutes. The volumes of the aliquots were reduced for analysis by SDS-PAGE (12.5% separation gel, 180V, 50 min). The SDS-PAGE gel was stained using Coomassie Blue overnight.

Isolation of His-Ubiquitin, *human recombinant* (6xHis-Ub) with Ni(II)-loaded nanoparticles: The Ni(II)-loaded nanoparticles (10 μL; 16.3 mg/mL) were washed in distilled water (3×50 μL) and wash buffer (3×50 μL; 50 mM phosphate buffer system, 300 mM NaCl, 10 mM imidazole, pH 8.0). The washed nanoparticles were placed in 40 uL of wash buffer and 10 uL of 6×His-Ub (1 μg 6×His-Ub/1 μL of distilled water) and incubated for 60 minutes at 4 °C. Using a centrifuge and magnet, the supernatant was collected and nanoparticles washed in 50 μL aliquots (FT, W1, W2, W3). After W3, 50 μL of elution buffer (50 mM phosphate buffer system, 300 mM NaCl, 250 mM imidazole, pH 8.0) was added and rotated for 10 minutes at 4°C. Nanoparticles were collected in 50 μL aliquots (E1, E2). Non-specifically bound protein was released from the nanoparticles by boiling at 95 °C for 4 minutes. The volumes of the aliquots were reduced for analysis by SDS-PAGE (12.5% separation gel, 180V, 50 min). The SDS-PAGE gel was stained using Coomassie Blue overnight.

Activity Assay of recombinant mouse His×6-Endostatin (His×6-rmES) eluted from Ni(II)-loaded nanoparticles. A previously published protocol is used to determine apoptotic activity of eluted Hisx6-rmES.(*1*) Briefly, cow pulmonary artery endothelial (CPAE) cells were maintained in DMEM containing 10% FCS, 2% L-glutamine, 1% penicillin/streptomycin at 37^{o}C and 5% CO_2. Prior to the induction of apoptosis, cells were transferred to DMEM supplemented with only 2% FCS and 3 ng/mL bFGF for 12 h. Cells were treated with His×6-rmES directly eluted from Ni(II)-loaded nanoparticles (10 μg/mL). As a negative control, cells were treated with an equivalent volume of elution buffer. Cells were collected by rapid trypsinization and washed twice with cold phosphate buffered saline (pH 7.4) before resuspension in binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$). Cells were incubated with Annexin V-PE following manufacturer's protocol. Stained cells were visualized using confocal fluorescence microscopy on a Leica SP2 Laser Scanning Confocal Microscope with 488 nm excitation and a 530 LP emission filter.

A List of Protein sequences, molecular weights, and calculated isoelectric points:

1. Mouse Endodtatin (N-terminal His-tagged)

```
        10         20         30         40         50         60
MARRASVGTD HHHHHHHTHQ DFQPVLHLVA LNTPLSGGMR GIRGADFQCF QQARAVGLSG

        70         80         90        100        110        120
TFRAFLSSRL QDLYSIVRRA DRGSVPIVNL KDEVLSPSWD SLFSGSQGQL QPGARIFSFD

       130        140        150        160        170        180
GRDVLRHPAW PQKSVWHGSD PSGRRLMESY CETWRTETTG ATGQASSLLS GRLLEQKAAS

       190        200
CHNSYIVLCI ENSFMTSFSK
```

Molecular weight: 22244.0

Theoretical pI: 9.33

2. Ubiquitin (N-terminal His-tagged)

```
        10         20         30         40         50         60
MHHHHHHQIF VKTLTGKTIT LEVEPSDTIE NVKAKIQDKE GIPPDQQRLI FAGKQLEDGR

        70         80
TLSDYNIQKE STLHLVLRLR GG
```

Molecular weight: 9387.6

Theoretical pI: 7.07

3. Mouse UCH-L1 (N-terminal His-tagged)

```
        10         20         30         40         50         60
MHHHHHHQLK PMEINPEMLN KVLAKLGVAG QWRFADVLGL EEETLGSVPS PACALLLLFP

        70         80         90        100        110        120
LTAQHENFRK KQIEELKGQE VSPKVYFMKQ TIGNSCGTIG LIHAVANNQD KLEFEDGSVL

       130        140        150        160        170        180
KQFLSETEKL SPEDRAKCFE KNEAIQAAHD SVAQKGQCRV DDKVNFHFIL FNNVDGHLYE

       190        200        210        220
LDGRMPFPVN HGASSEDSLL QDAAKVCREF TEREQGEVRF SAVALCKAA
```

Molecular weight: 25660.1

Theoretical pI: 5.83

4. C. elegans UGT (C-terminal His-tagged)

```
        10         20         30         40         50         60
MLLRILTFLA VCQVTTSHKI LMFSPTASKS HMISQGRIAD ELANAGHEVV NFEPDFLNLT

        70         80         90        100        110        120
```

```
DKFVPCKKCR RWPVTGLNNY KFKKIQNGLS GDVFQQSSIW SKIFNTDSDP YQDEYTNMCE

       130        140        150        160        170        180
EMVTNKELIE KLKKEKFDAY FGEQIHLCGM GLAHLIGIKH RFWIASCTMS VSMRDSLGIP

       190        200        210        220        230        240
TPSSLIPFMS TLDATPAPFW QRAKNFVLQM AHIRDEYRDV VLTNDMFKKN FGSDFPCVEF

       250        260        270        280        290        300
LAKTSDLIFV STDELLEIQA PTLSNVVHIG GLGLSSEGGG LDEKFVKIME KGKGVILFSL

       310        320        330        340        350        360
GTIANTTNLP PTIMENLMKI TQKFKDYEFI IKVDKFDRRS FDLAEGLSNV LVVDWVPQTA

       370        380        390        400        410        420
VLAHPRLKAF ITHAGYNSLM ESAYAGVPVI LIPFMFDQPR NGRSVERKGW GILRDRFQLI

       430        440        450        460        470        480
KDPDAIEGAI KEILVNPTYQ EKANRLKKLM RSKPQSASER LVKMTNWVLE NDGVEELQYE

       490        500        510        520        530        540
GKHMDFFTFY NLDIIITAAS IPVLIFIVLR ISNISIITSS PKNKKDKGQD NSADIQHSGG

       550        560        570
RSSLEGPRFE GKPIPNPLLG LDSTRTGHHH HHH
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Molecular weight: 64722.7

Theoretical pI: 8.03

5. GMAP (C-terminal His-tagged)

```
M Q L L F T I T M E K G E I E A E L C W A K K R L L E E A N K Y E K T I
E E L S N A R N L N T S A L Q L E H E H L I K L N Q K K D M E I A E L K
K N I E Q M D T D H K E T K D V L S S S L E E Q K Q L T Q L I N K K E I
F I E K L K E R S S K L Q E E L D K Y S Q A L R K N E I L R Q T I E E K
D R S L G S M K E E N N H L Q E E L E R L R E E Q S R T A P V A D P K T
L D S V T E L A S E V S Q L N T I K E H L E E E I K H H Q K I I E D Q N
Q S K M Q L L Q S L Q E Q K K E M D E F R Y Q H E Q M N A T H T Q L F L
E K D E E I K S L Q K T I E Q I K T Q L H E E R Q D I Q T D N S D I F Q
E T K V Q S L N I E N G S E K H D L S K A E T E R L V K G I K E R E L E
I K L L N E K N I S L T K Q I D Q L S K D E V G K L T Q I I Q Q K D L E
I Q A L H A R I S S T S H T Q D V V Y L Q Q Q L Q A Y A M E R E K V F A
V L N E K T R E N S H L K T E Y H K M M D I V A A K E A A L I K L Q D E
N K K L S T R F E S S G Q D M F R E T I Q N L S R I I R E K D I E I D A
L S Q K C Q T L L A V L Q T S S T G N E A G G V N S H Q F E E L L Q E R
D K L K Q Q V K K M E E W K Q Q V M T T V Q N M Q H E S A Q L Q E E L H
Q L Q A Q V L V D S D N N S K L Q V D Y T G L I Q S Y E Q N E T K L K N
F G Q E L A Q V Q H S I G Q L C N T K D L L L G K L D I I S P Q L S S A
S L L T P Q S A E C L R A S K S E V L S E H H H H H H
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Number of amino acids: 639

Molecular weight: 74406.8

Theoretical pI: 5.43

Table S1. Radioactivity counts in counts per minute (CPM) for different fractions of S^{35}-labeled 6xHis-GMAP binding scintillation counting assay

	Blank (CPM)	**DL** (CPM)	**FT** (CPM)	**W1** (CPM)	**W2** (CPM)	**W3** (CPM)	**E1** (CPM)	**E2** (CPM)	**Resin** (CPM)
Bis-Ni-NTA-NP	38	1685170	1636552	39410	1980	653	3840	764	1971
Commercial Beads	85	1685170	1500000	60783	490	248	3602	430	369

DL = Direct Lysate, FT = Flow Through, W1 = Wash 1, W2 = Wash 2, W3 = Wash 3, E1 = Elution 1, E2 = Elution 2.

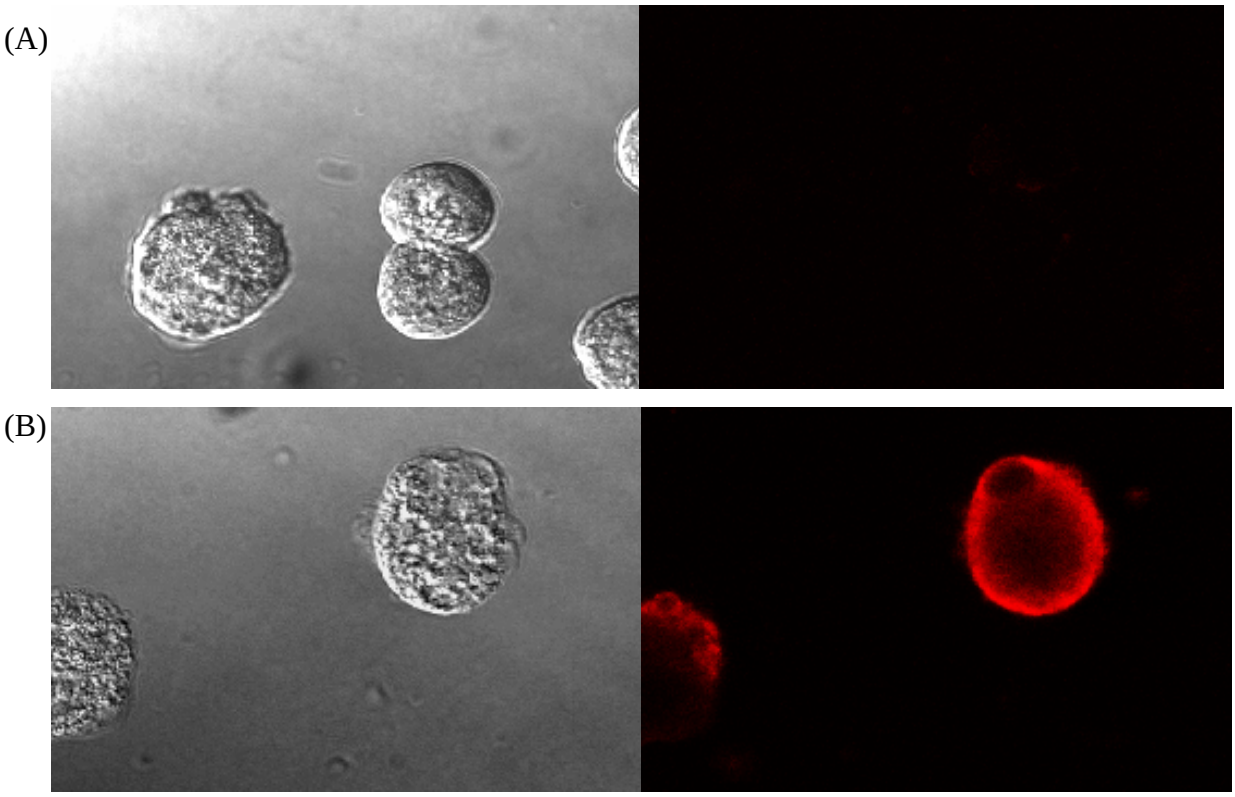


Figure S1. (A) Bright field (left) and corresponding confocal fluorescence microcopy (right) images of normal cow pulmonary artery endothelial (CPAE) cells (without 6×His-rmES treatment). (B) Bright field (left) and corresponding confocal fluorescence microcopy (right) images of apoptotic CPAE cells labeled with Annexin V-PE. Apoptosis was induced over 24 hours using 6×His-rmES (10 μg/mL) eluted from bis-Ni-NTA nanoparticles.

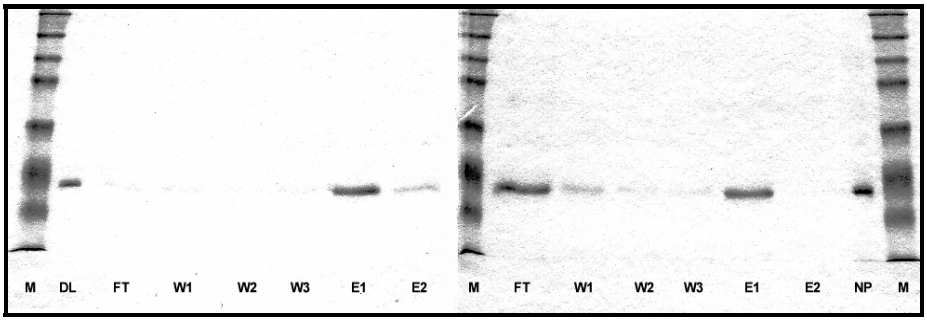


Figure S2. SDS-PAGE analysis of 6xHis-rmES binding assay with Ni(II)-loaded nanoparticles. (Left), with pristine nanoparticles; (Right) with recycled nanoparticles. The two merged gels show similar binding capacities for eluted protein (E1).

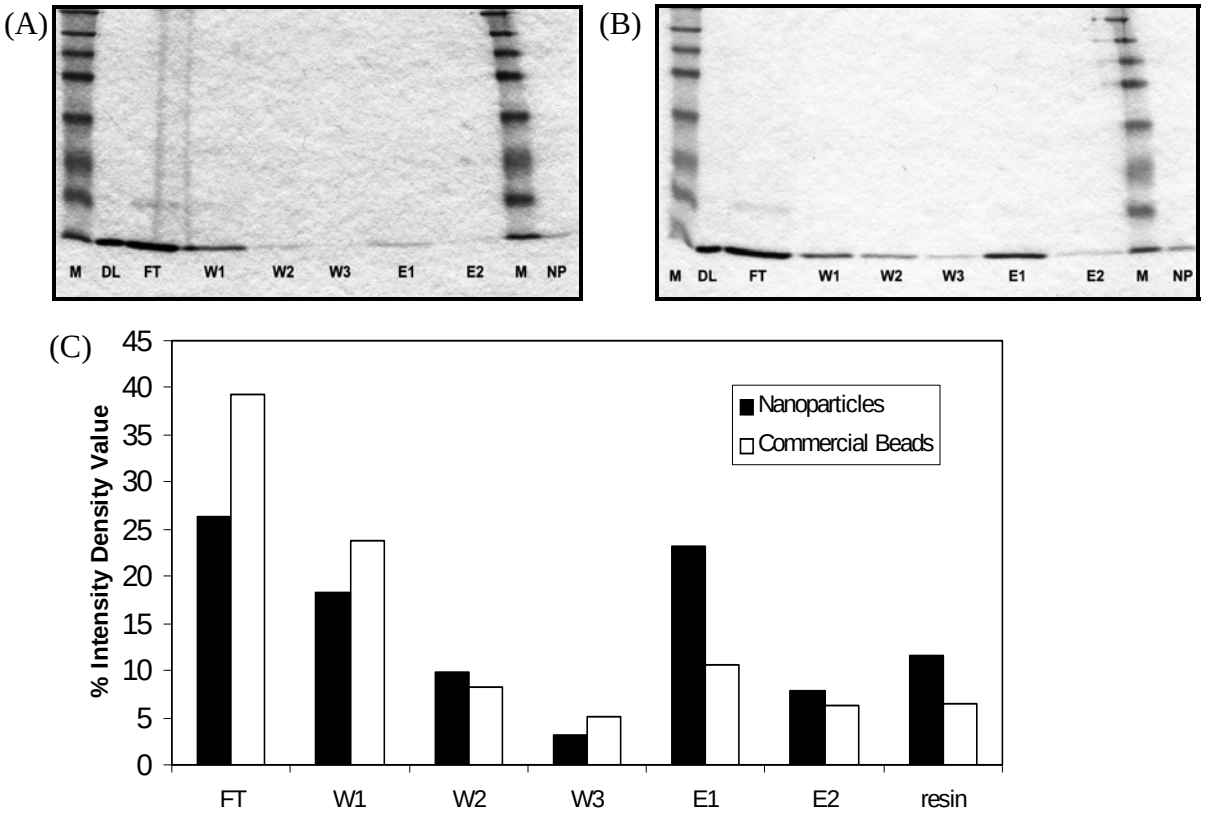


Figure S3. Binding assay of 6×His-Ub. (A) Commercial magnetic agarose beads (Qiagen): no protein is bound to the bead or eluted by imidazole. Most of the protein is washed away unbound in the FT. (B) Bis-Ni-NTA NPs: His-tag protein is bound and a majority is eluted from the nanoparticles by imidazole. (C) Graphical representation of densitometry quantification of SDS-PAGE gels.

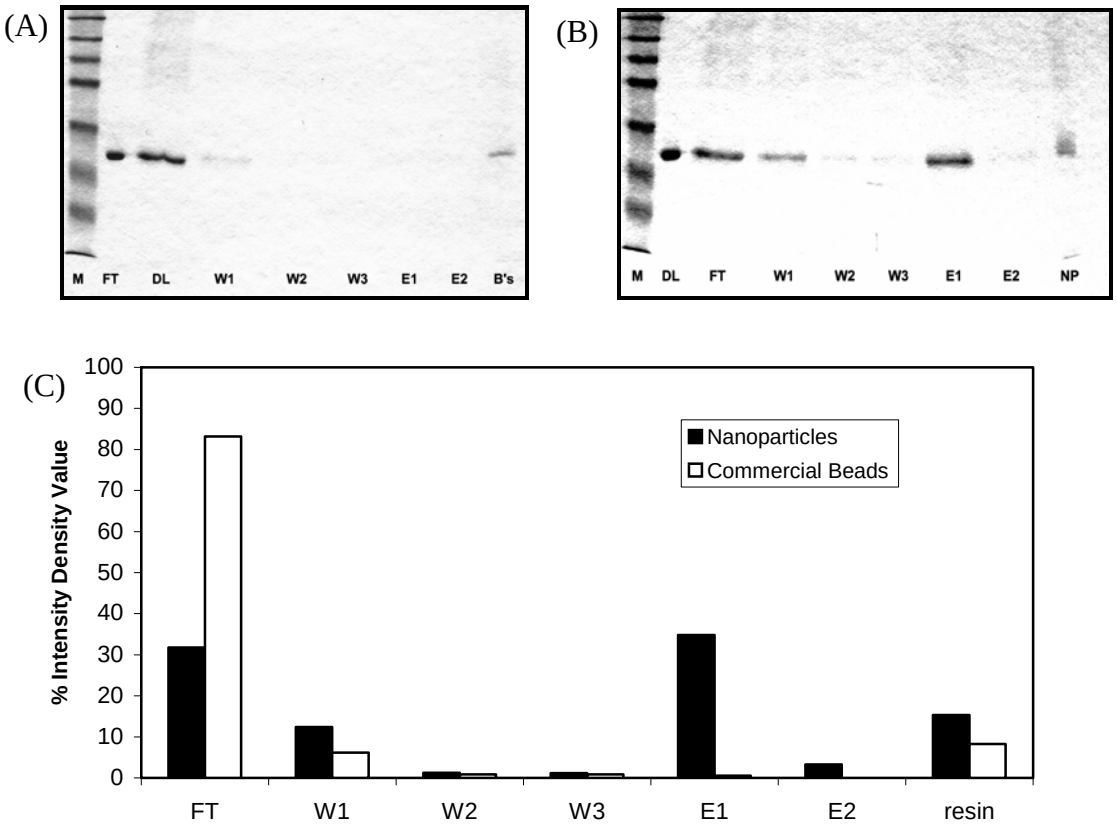


Figure S4. Binding assay of 6xHis-UCH-L1. (A) Commercial magnetic agarose beads (Qiagen): no enzyme is bound to the bead or eluted by imidazole. Most of the enzyme is washed away unbound in the FT. (B) Bis-Ni-NTA NPs: His-tag enzyme is bound and a majority is eluted from the nanoparticles by imidazole. (C) Graphical representation of densitometry quantification of SDS-PAGE gels.

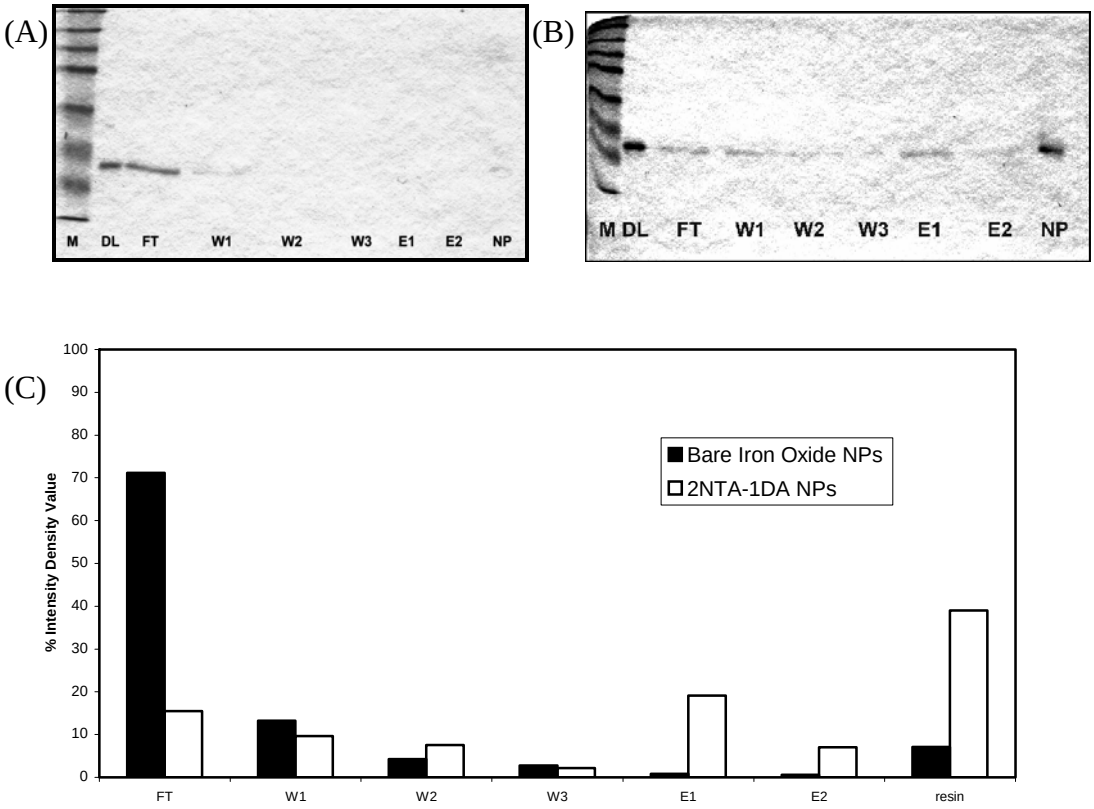
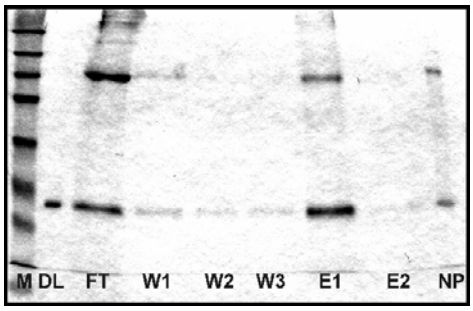


Figure S5. Interaction of 6xHis-rmES with (A) bare iron oxide nanoparticles and (B) ligand modified nanoparticles. Graphical representation of densitometry quantification of SDS-PAGE gels is shown in (C).

(A)



(B)

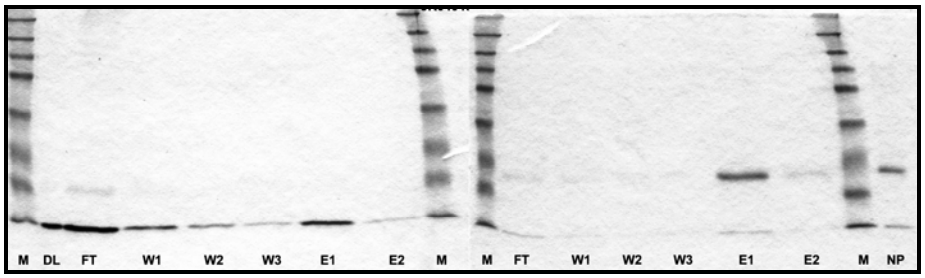


Figure S6. Attempts at blocking nonspecific interaction occurring in 6xHis-rmES binding assay. (A) Ineffective blocking shown in SDS-PAGE analysis after overnight pre-incubation of nanoparticles with 1% BSA. The upper lane is due to BSA. (B) Ineffective blocking shown in SDS-PAGE analysis after recycling nanoparticles used in 6xHis-Ub binding assay for a 6xHis-rmES binding assay. Left, 6xHis-Ub binding assay; Right, 6xHis-rmES binding assay with recycled nanoparticles.

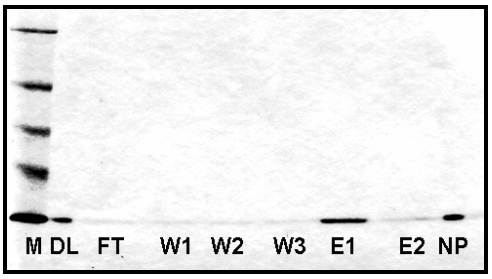
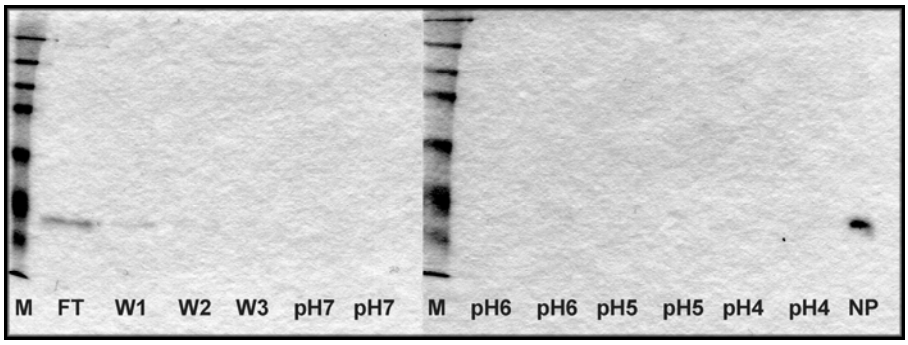


Figure S7. 6xHis-rmES binding assay using mono-Ni-NTA nanoparticles demonstrate similar binding capacity as the bis-Ni-NTA nanoparticles.

(A)



(B)

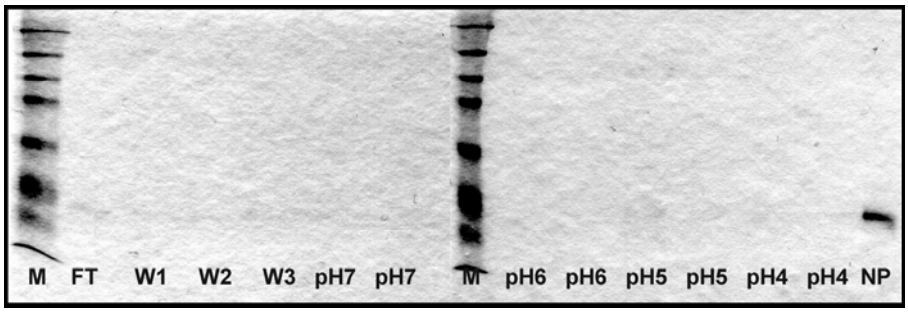


Figure S8. Attempt toward pH elution in 6xHis-rmES binding assay for (A) bis-Ni-NTA nanoparticles and (B) mono-Ni-NTA nanoparticles showing no elution of protein at pH of 7, 6, 5, and 4 (washed twice at each pH) without the presence of imidazole. All of the protein remains on the nanoparticles.

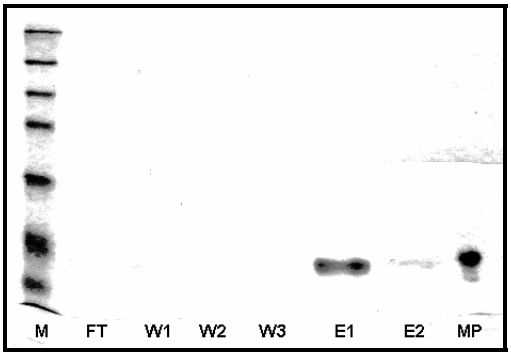


Figure S9. 6xHis-rmES binding assay using Ni(II)-NTA conjugated microparticles (MP) demonstrating similar binding capacity as bis-Ni-NTA nanoparticles by high surface coverage.

(*1*) Dhanabal, M., Ramchandran, R., Waterman, M.J., Lu, H., Knebelmann, B., Segal, M., Sukhatme, V.P. (1999) Endostatin induces endothelial cell apoptosis. *J. Biol. Chem. 274*, 11721-11726.