

## Supplementary Materials

### A Spy Chemistry-Based Method for Purification of Proteins with Native N-Termini

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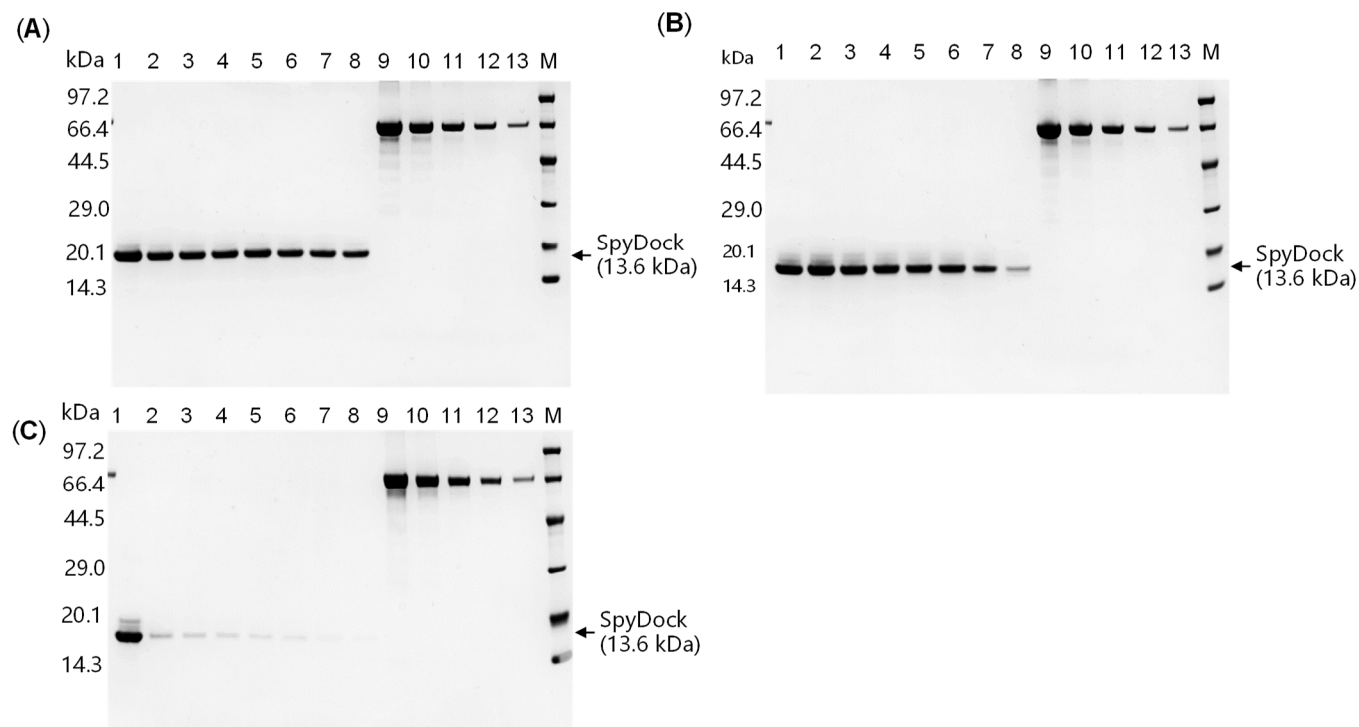
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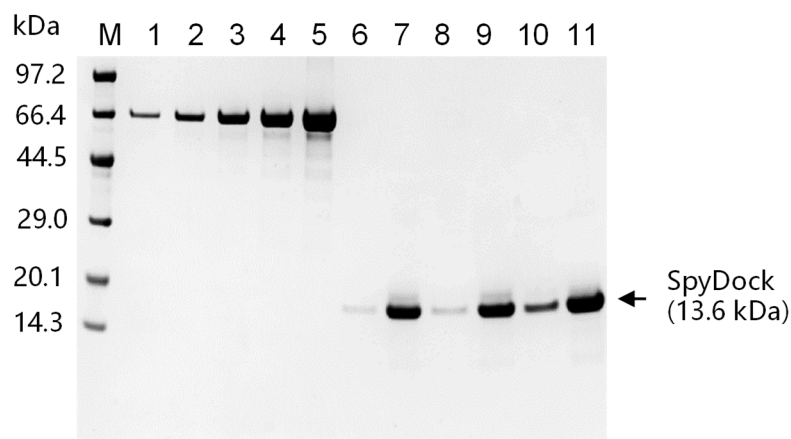
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1. Supplemental Figures S1-S4
2. Supplemental Table S1-S2

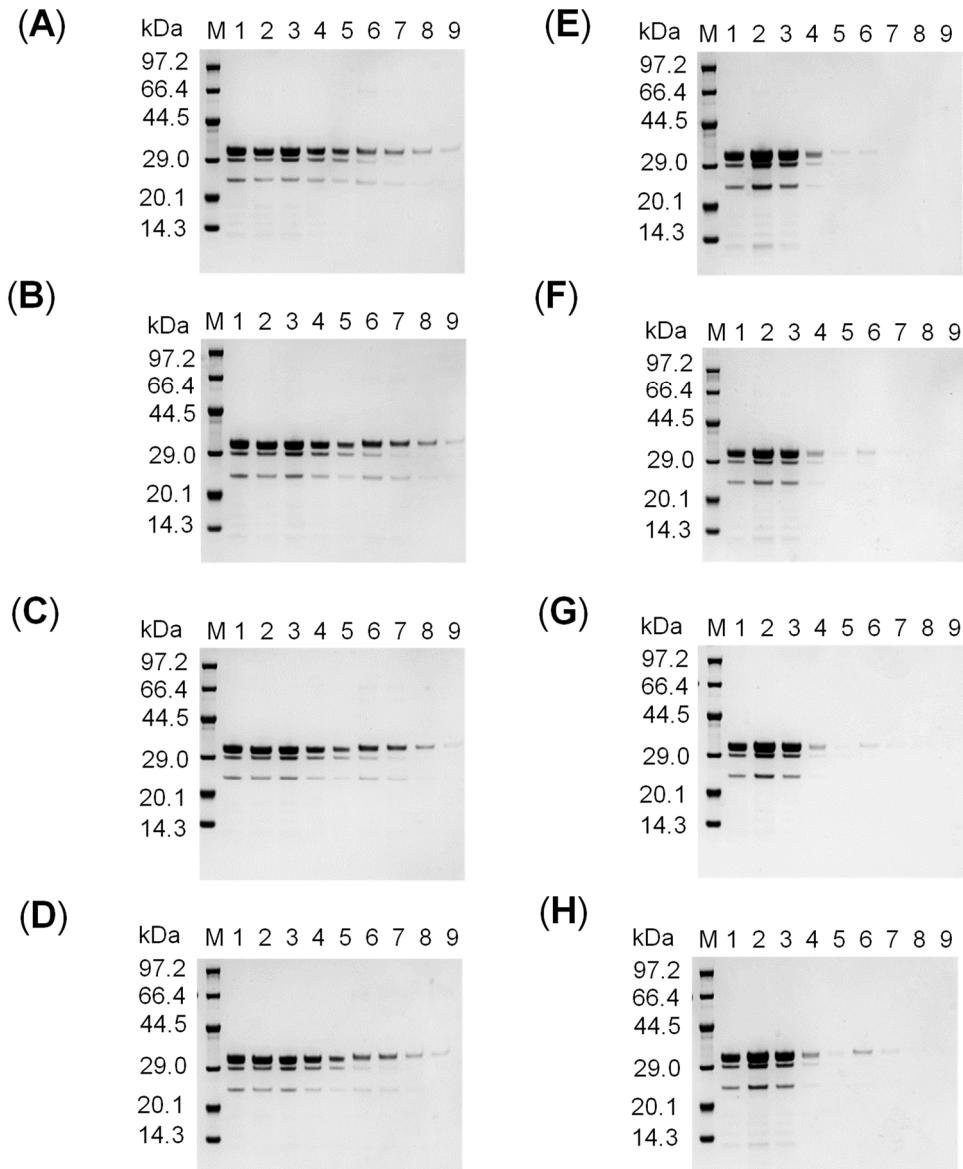
## 1. Supplemental Figures



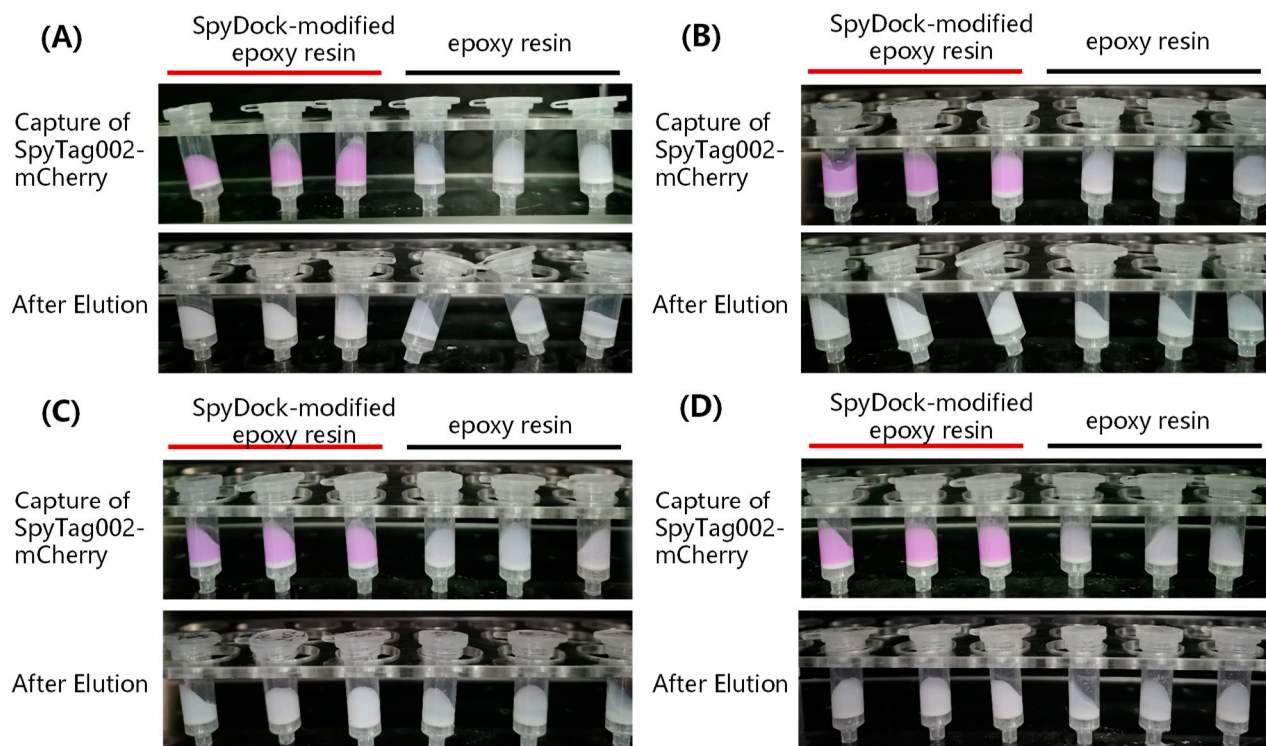
**Figure S1. SDS-PAGE results depicting the immobilization of SpyDock on epoxy resin at different pH levels:** (A) pH = 7.0, (B) pH = 8.0, (C) pH = 10.0. Lane 1: SpyDock before immobilization. Lanes 2-8: SpyDock supernatant after immobilization for 2, 4, 6, 8, 10, 12, and 24 hours. Lanes 9-13: Bovine serum albumin (BSA) standard solutions (from left to right: 0.03125 mg/mL, 0.0625 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.50 mg/mL). M: molecular weight marker.



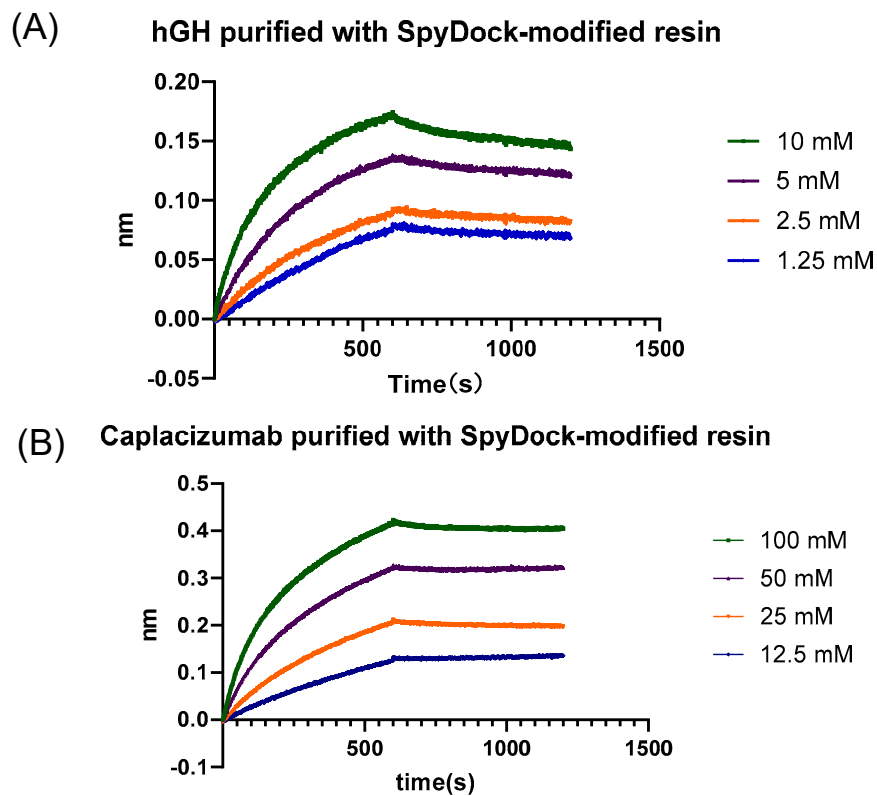
**Figure S2. SDS-PAGE results for the immobilization of SpyDock on epoxy resin with varying input amounts.** M: molecular weight marker. Lanes 1-5: BSA standard solutions (from left to right, 0.03125 mg/mL, 0.0625 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.50 mg/mL). Lanes 6, 8, 10: Remaining SpyDock in the supernatant after immobilization on the epoxy resin. Lanes 7, 9, 11: SpyDock input for immobilization. The input amounts of SpyDock are 20 mg/mL for lanes 6 and 7, 60 mg/mL for lanes 8 and 9, and 100 mg/mL for lanes 10 and 11.



**Figure S3. SDS-PAGE results demonstrating the reusability of SpyDock-modified resin and initial epoxy resin.** (A-D) The first, second, third and fourth rounds of using SpyDock-modified resin for purifying SpyTag002-RFP; (E-H) The first, second, third and fourth round of use of initial epoxy resin for purifying SpyTag002-RFP. M: molecular weight marker; Lane 1: SpyTag002-RFP loaded; Lane 2: SpyTag002-RFP in flow-through; Lane 3-5: SpyTag002-RFP washed with TP buffer; Lane 6-9: SpyTag002-RFP eluted with ETP buffer.



**Figure S4. Photographs for the (A) first, (B) second, (C) third, and (D) fourth rounds of using SpyDock-modified resin for purifying SpyTag002-RFP.**



**Figure S5** Binding of hGH and Caplacizumab monitored with BLI. **(A)** hGH purified with SpyDock-modified resin. **(B)** Caplacizumab purified with SpyDock-modified resin.

## 2. Supplemental Table

**Table S1. Primers used in this study.**

Primer name	Sequence (5'-3')	Description
SpyDock-F	CTCGATCCCGCGAAATTAATAC GACTC	SpyDock fragment for the construction of pET30a-SpyDock
SpyDock-R	CACGTATGTGCATCGCCTTCGG	
BackboneS-F	GAAGGCGATGCACATACGTGA GATCCGGCTGCTAACAAAGCC CGAAAG	Backbone for the construction of pET30a- SpyDock
BackboneS-R	TGAGTCGTATTAATTCGCGGG ATCGAGATC	
RFP-F-1	GTGCCTACTATTGTGATGGTGG ACGCCTACAAGCGTTACAAAG GCGGTGGCGGCAGCGG	SpyTag002-Gslinker- RFP fragment for the construction of pET30a- SpyTag002-Gslinker- RFP
RFP-F-2	TTAACTTTAAGAAGGAGATATA CATATGGTGCCTACTATTGTGAT GGTGGACG	
RFP-R	GGCAAGTGTAGCGGTCACGC	
Backbone-F	GCGTGACCGCTACACTTGCC	Backbone for the construction of pET30a- SpyTag002-Gslinker- RFP
Backbone-R	CATATGTATATCTCCTTCTTAAA GTTAAACAAAATTATTTCTAGA GGG	
hGH-F-1	GTGCCTACTATTGTGATGGTGG ACGCCTACAAGCGTTACAAAG GCGGTGGCGGCAGCGG	SpyTag002- <i>Mtu</i> $\Delta$ I-CM- hGH fragment for the construction of pET30a- Spytag- <i>Mtu</i> $\Delta$ I-CM- hGH
hGH-F-2	GGAATTCCATATGGTGCCTACT ATTGTGATGGTGGACGC	
hGH-R	CCGCTCGAGGAAACCGCAAGA ACCTTCAACAGAACGGC	
GST-F	CGACGCACCGCATTGAAGATG TTG	<i>Mtu</i> $\Delta$ I-CM -GST fragment for the construction of pET30a- SpyTag002- <i>Mtu</i> $\Delta$ I-CM - GST
GST-R	GTGGTGGTGGTGGTGCCTCGAG TTTTGGAGGATGGTCGCCACCA CCAAACGTGGCTTG	

Caplacizumab-F	CGACGCACCGCATTGAAGATG TTG	Caplacizumab fragment for the construction of
Caplacizumab-R	TCGCCAATCCGGATATAGTTCC TCC	pET30a- SpyTag002- <i>Mtu</i> $\Delta$ I-CM-Caplacizumab
GST-F-N	ATGTCCCCTATACTAGGTTATTG GA	GST-His fragment for the construction of
GST-R-N	GTGGTGGTGGTGGTGCTCGAG TTTTGGAGGATGGTCGCCACCA CCAAACGTGGCTTG	pET30a-GST-His
hGH-F-N	TAATTTTGTTTAACTTTAAGAA GGAGATATACATATGTTCCCGA CCATCCCGCTGTC	hGH-His fragment for the construction of
hGH-R-N	CGAGGTATGTAGGCGGTGCTAC	pET30a-hGH-His
Caplacizumab-F-N	CACCAGTGAGACGGGCAACA GC	Caplacizumab-His fragment for the
Caplacizumab-R-N	GTGGTGGTGGTGGTGCTCGAGGCT AGAAACGGTAACCTGGGTG	construction of pET30a- Caplacizumab-His



**Table S2. Binding capacity and elution recovery of SpyDock-modified epoxy resin.**

<b>SpyTag002- RFP loaded (mg/mL resin)</b>	<b>SpyTag002- RFP captured (mg/mL resin)</b>	<b>Capture efficiency (%)</b>	<b>SpyTag002- RFP eluted (mg/mL resin)</b>	<b>Elution volumes</b>	<b>Elution recovery efficiency (%)</b>
5	3.3±0.5	66±10	2.6±0.1	4 ×1.5 resin volume	81±12
10	7.3±0.1	73±0.1	3.7±0.1	4 ×1.5 resin volume	50±3
30	22.7±4	73±5	17.0±3.6	8 ×1.5 resin volume	76±13