

## Assessing histidine tags for directing deoxyribozyme-catalyzed peptide and protein modification reactions

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Oligonucleotides and oligonucleotide-tris(NTA) conjugates*Sequences of oligonucleotides for DNA-catalyzed peptide-RNA conjugation*

oligonucleotide purpose	oligonucleotide sequence, 5' to 3'
8XJ105 deoxyribozyme <sup>a</sup>	CCCGAAAGCCTCCTTC <u>GGGAGATGTCTCTCAGACGGAAACTTTCAGTACGGAATGG</u> ATACGCATAAAGGTAG
3'-tris(NTA) anchor DNA <sup>b</sup>	CTACCTTTATGCGTAT-HEG-XXX
5'-tris(NTA) anchor DNA <sup>c</sup>	XXXXXXXXXXXXXXXXXXXXTACCTTTATGCGTAT
control anchor DNA	AACAACAACAACAACAACAACGGACTACCTTTATGCGTAT
5'-pppRNA substrate	ppp-r (GGAAGGAGGCUUUCGGG)
decoy oligo for assay of 8XJ105 <sup>d</sup>	TTATGCGTAT <u>CCATTCCGTA</u> CTGAAAGTTTCCGCTCTGAGAGACATCTCCC GAAGGAGGCT

*Sequences of oligonucleotides for DNA-catalyzed tyrosine phosphorylation*

oligonucleotide purpose	oligonucleotide sequence, 5' to 3'
6CF134 deoxyribozyme <sup>e</sup>	GAAGCGCTAGAACAT <u>GGGGACAGGCAGCTCCACCGATGGGCACCG</u> ATAGTGAGTCGTATT
3'-tris(NTA) anchor DNA <sup>b</sup>	TAATACGACTCACTAT-HEG-XXX
control anchor DNA	GGATAATACGACTCACTAT
5'-pppRNA substrate	ppp-r (GAUGUUCUAGCGCUUCG)

**Table S1.** Oligonucleotides used during the experiments. All sequences are written 5' to 3' and are DNA unless explicitly indicated as RNA.

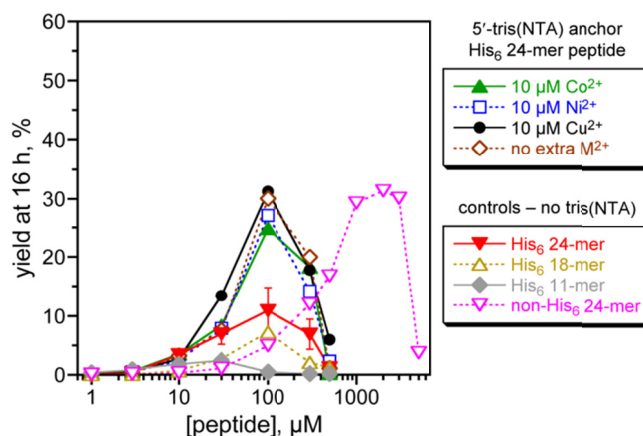
<sup>a</sup> The underlined nucleotides are the initially random (N<sub>40</sub>) region of 8XJ105. The non-underlined nucleotides are the binding arms.

<sup>b</sup> “HEG” is the hexa(ethylene glycol) spacer provided as Spacer 18 (Glen Research). X is the Uni-Link Amino Modifier (Clontech).

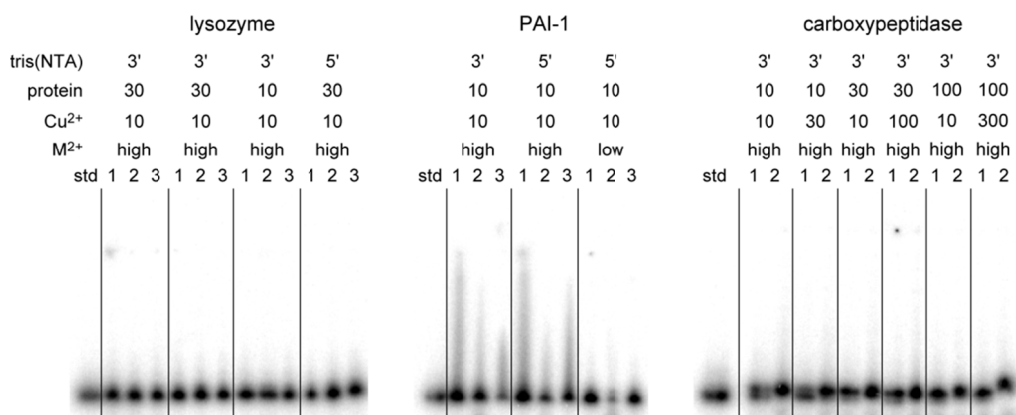
<sup>c</sup> X is the Uni-Link Amino Modifier (Clontech). See Fig. 2A for structure of this modifier after conversion to tris(NTA) derivative.

<sup>d</sup> The underlined nucleotides are complementary to the initially random (N<sub>40</sub>) region of 8XJ105. The non-underlined nucleotides are complementary to the flanking sequences of the binding arms.

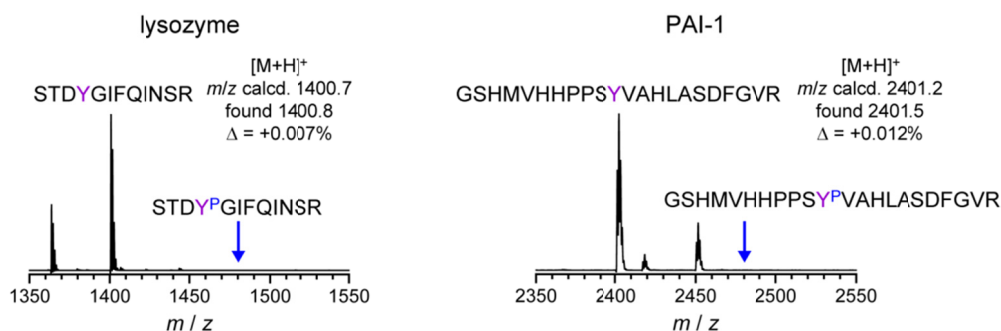
<sup>e</sup> The underlined nucleotides are the initially random (N<sub>30</sub>) region of 6CF134. The non-underlined nucleotides are the binding arms.

Assessing histidine tag recruiting for 8XJ105 with 5'-tris(NTA) DNA anchor

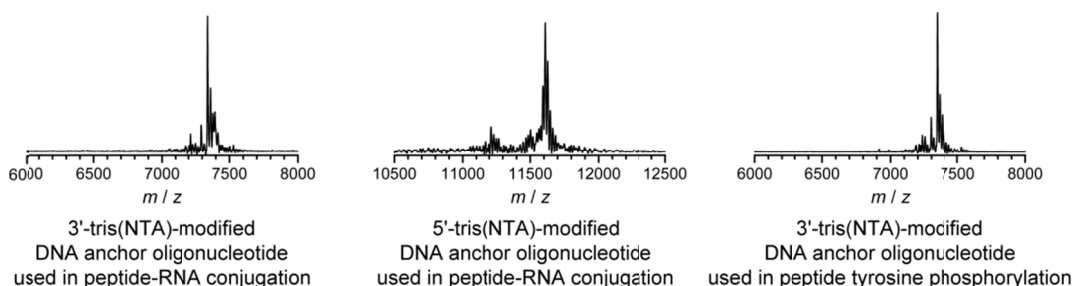
**Figure S1.** Data analogous to that in Fig. 3A, here for the 5'-tris(NTA) anchor oligonucleotide.

SDS-PAGE assays of 8XJ105 with protein substrates

**Figure S2.** SDS-PAGE assays of 8XJ105 with the three protein substrates, lysozyme (15.2 kDa), plasminogen activator inhibitor 1 (PAI-1; 45 kDa), and carboxypeptidase B (34.5 kDa). std = pppRNA. The location of the tris(NTA) moiety, either 3' or 5' on the DNA anchor oligonucleotide, is shown. Concentrations of protein and Cu<sup>2+</sup> are shown in  $\mu$ M. Divalent metal ions (M<sup>2+</sup>) were either "low" (1 mM Mg<sup>2+</sup>, 5 mM Mn<sup>2+</sup>, and 0.5 mM Zn<sup>2+</sup>) or "high" (40 mM Mg<sup>2+</sup>, 20 mM Mn<sup>2+</sup>, and 1 mM Zn<sup>2+</sup>). The incubation conditions labeled as 1, 2, and 3 over individual lanes were as follows: 1 = room temperature, 30 s; 2 = room temperature, 16 h; 3 = 4 °C, 16 h. In all cases, no protein-RNA conjugate product was observed, as revealed by the absence of any slower-migrating band higher on the gel image. Not depicted for each gel image is a nonradiolabeled protein ladder (Bio-Rad cat. no. 161-0318, 7–209 kDa), which was included to calibrate our expectation for the position of the product band on the 16% Tris-Tricine SDS-PAGE gel.

Trypsin/MALDI mass spectrometry assays of 6CF134 with protein substrates

**Figure S3.** Representative trypsin/MALDI mass spectrometry assays of 6CF134 with the two protein substrates, lysozyme (15.2 kDa) and plasminogen activator inhibitor 1 (PAI-1; 45 kDa). Tryptic fragments were assigned with confidence in 5 of 6 instances for lysozyme and 2 of 5 instances for PAI-1. In each of these cases, no phosphorylation product peak was observed.

Mass spectra of tris(NTA)-modified DNA oligonucleotides

**Figure S4.** Mass spectra of the three tris(NTA)-modified DNA oligonucleotides. The detailed synthesis procedures and quantitative information are given in the Experimental section.