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 [a]	CCCGAAAGCCTCCTTCGGGAGATGTCTCTCAGACGGAAACTTTCAGTACGGAATGG ATACGCATAAAGGTAG
3′-tris(NTA) anchor DNA [b]	CTACCTTTATGCGTAT–HEG–XXX
5′-tris(NTA) anchor DNA [c]	XXXTTTTTTTTTTTTTTTCTACCTTTATGCGTAT
control anchor DNA	AACAACAACAACAACAACAACGGACTACCTTTATGCGTAT
5′-pppRNA substrate	ppp-r(GGAAGGAGGCUUUCGGG)
decoy oligo for assay of 8XJ105 [d]	TTATGCGTATCCATTCCGTACTGAAAGTTTCCGTCTGAGAGACATCTCCC GAAGGAGGCT

Sequences of oligonucleotides for DNA-catalyzed tyrosine phosphorylation

oligonucleotide purpose	oligonucleotide sequence, 5′ to 3′
6CF134 deoxyribozyme [e]	GAAGCGCTAGAACATGGGGACAGGCAGCTCCACCGATGGGCACCG ATAGTGAGTCGTATT
3′-tris(NTA) anchor DNA [b]	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.

[a] The underlined nucleotides are the initially random (N_{40}) region of 8XJ105. The non-underlined nucleotides are the binding arms.

[b] "HEG" is the hexa(ethylene glycol) spacer provided as Spacer 18 (Glen Research). X is the Uni-Link Amino Modifier (Clontech).

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

[d] The underlined nucleotides are complementary to the initially random (N_{40}) region of 8XJ105. The non-underlined nucleotides are complementary to the flanking sequences of the binding arms.

[e] The underlined nucleotides are the initially random (N_{30}) region of 6CF134. The non-underlined nucleotides are the binding arms.

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

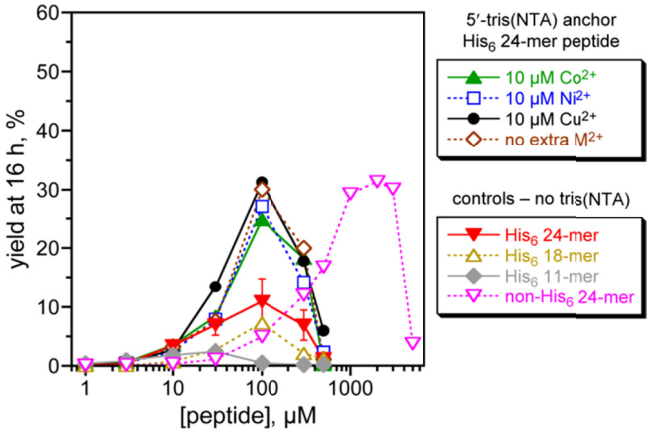

Figure S1. Data analogous to that in Fig. 3A, here for the 5′-tris(NTA) anchor oligonucleotide.

SDS-PAGE assays of 8XJ105 with protein substrates

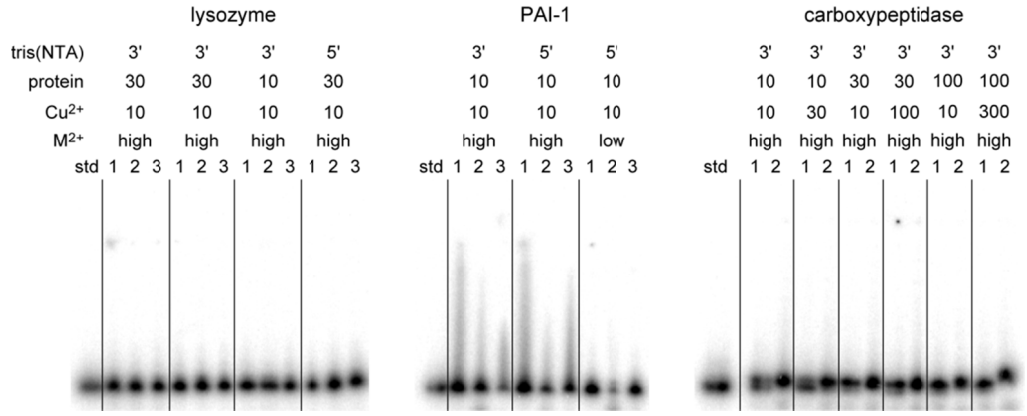

Figure S2. SDS-PAGE assays of 8XJ105 with the three protein substrates, lysozyme (15.2 kDa), plasminogen activator inhibitor 1 (PAI-1; 45 kDa), and carboxypeptidase B (34.5 kDa). std = pppRNA. The location of the tris(NTA) moiety, either 3′ or 5′ on the DNA anchor oligonucleotide, is shown. Concentrations of protein and Cu^{2+} are shown in μM. Divalent metal ions (M^{2+}) were either "low" (1 mM Mg^{2+}, 5 mM Mn^{2+}, and 0.5 mM Zn^{2+}) or "high" (40 mM Mg^{2+}, 20 mM Mn^{2+}, and 1 mM Zn^{2+}). 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

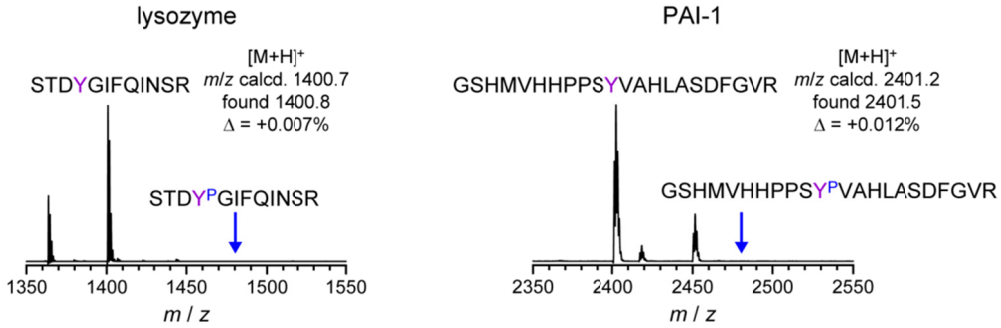

Figure S3. Representative trypsin/MALDI mass spectrometry assays of 6CF134 with the two protein substrates, lysozyme (15.2 kDa) and plasminogen activator inhibitor 1 (PAI-1; 45 kDa). Tryptic fragments were assigned with confidence in 5 of 6 instances for lysozyme and 2 of 5 instances for PAI-1. In each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

Mass spectra of tris(NTA)-modified DNA oligonucleotides

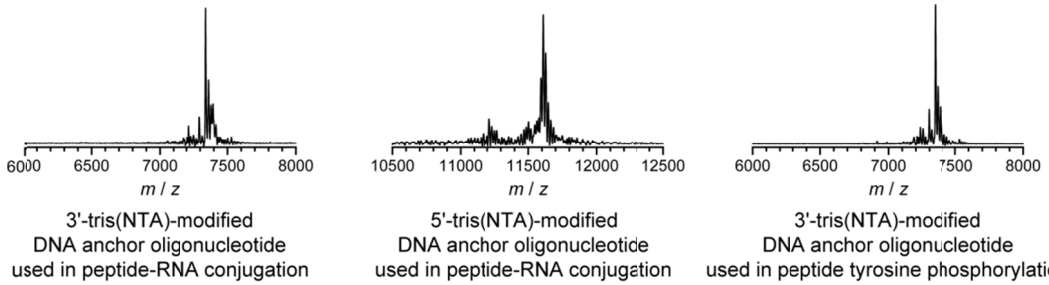

Figure S4. Mass spectra of the three tris(NTA)-modified DNA oligonucleotides. The detailed synthesis procedures and quantitative information are given in the Experimental section.