Organic & Biomolecular Chemistry

Supporting Information

Oligonucleotide modifications enhance probe stability for single cell transcriptome in vivo analysis (TIVA)

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Contents:

**Figure S1**
RP-HPLC purification of crude 22/9/9 GC probe after cleavage

**Figure S2**
AX-HPLC purification of 22/9/9 GC probe after conjugation to (D-Arg)\textsubscript{9} cell-penetrating peptide

**Figure S3**
RP-HPLC purification of crude PS-22/9/9 probe after solid-phase synthesis and cleavage

**Figure S4**
FRET efficiencies of 18/7/7 and 22/9/9 TIVA probes in buffer, pre-photolysis

**Figure S5**
RP-HPLC purification of crude 22/9/9 GC probe after solid-phase synthesis and cleavage

**Figure S6**
AX-HPLC purification of 22/9/9 probe after conjugation to (D-Arg)\textsubscript{9} cell-penetrating peptide

**Figure S5**
RP-HPLC purification of cleaved 18/7/7 TIVA syntheses before and after protocol improvements

**Figure S8**
ESI-MS analysis of 22/9/9 + (D-Arg)\textsubscript{9} TIVA
**Figure S1.** RP-HPLC purification of crude 22/9/9 GC probe after solid-phase synthesis and cleavage

Separation was performed on C-18 column under a gradient of increasing acetonitrile in 0.5 M TEAA, with the product eluting at roughly 28 min.
**Figure S2.** AX-HPLC purification of 22/9/9 GC probe after conjugation to (D-Arg)$_9$ cell-penetrating peptide

Separation was performed on Source 15q ion-exchange column under a gradient of increasing NaClO$_4$ in 1:1 formamide:Tris-HCl buffer, with the product eluting at roughly 24 min.
**Figure S3.** RP-HPLC purification of crude PS-22/9/9 probe after solid-phase synthesis and cleavage

Separation was performed on C-18 column under a gradient of increasing acetonitrile in 0.5 M TEAA, with the product eluting at roughly 27 min.
Figure S4. FRET efficiencies of 18/7/7 and 22/9/9 TIVA probes in buffer, pre-photolysis

FRET efficiencies were measured for both probes at 1.0 μM in 1x STE buffer. 18/7/7 and 22/9/9 TIVA probes were synthesized according to [19].
Figure S5. RP-HPLC purification of crude 22/9/9 GC probe after solid-phase synthesis and cleavage

Separation was performed on C-18 column under a gradient of increasing acetonitrile in 0.5 M TEAA, with the product eluting at roughly 50 min.
Figure S6. AX-HPLC purification of 22/9/9 probe after conjugation to (D-Arg)$_3$ cell-penetrating peptide

Separation was performed on Source 15q ion-exchange column under a gradient of increasing NaClO$_4$ in 1:1 formamide:Tris-HCl buffer, with the product eluting at roughly 24 min.
**Figure S7.** RP-HPLC purification of cleaved 18/7/7 TIVA syntheses before and after protocol improvements

RP-HPLC purification of six different 18/7/7 TIVA probe syntheses, three before (A) and three after (B) protocol improvements, resulting in more consistent syntheses with higher yield. Separation was performed on a C-18 column under a gradient of increasing acetonitrile in 0.5 M.
Figure S8. ESI-MS analysis of 22/9/9 +(D-Arg)₉ TIVA

ESI-MS verified the product mass (16,913 Da predicted, 16,912 Da observed). The principal impurity corresponded to TIVA product with one missing 2' F-U (-307 Da), which is not expected to significantly impact probe performance.