Organic & Biomolecular Chemistry

Supporting Information

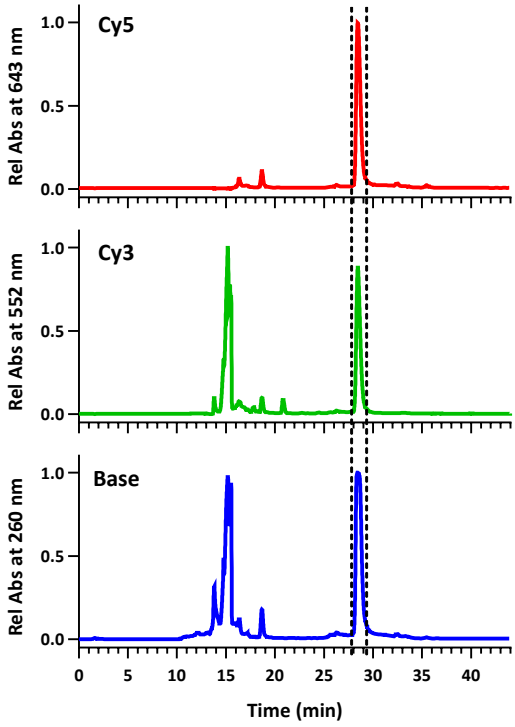
Oligonucleotide modifications enhance probe stability for single cell transcriptome in vivo analysis (TIVA)

S. B. Yeldell[a], B. K. Ruble[a], and I. J. Dmochowski[a]

[a]Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, PA 19104-6323, USA. E-mail: ivandmo@sas.upenn.edu
Tel: +1 215-898-6459

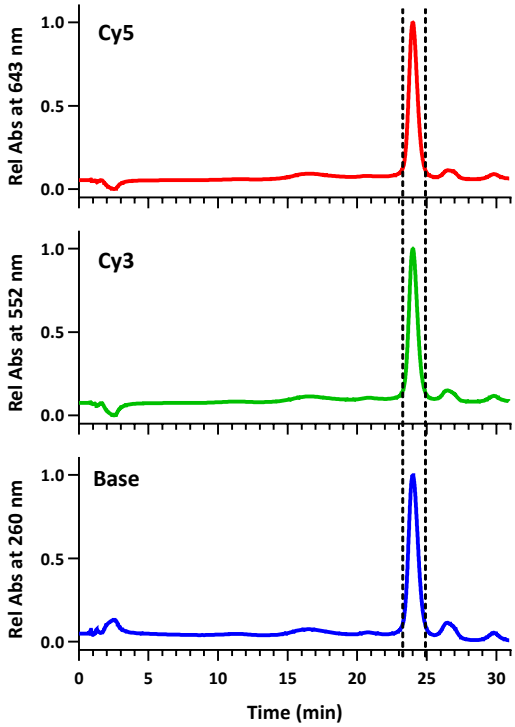
Contents:

Figure S1. RP-HPLC purification of crude 22/9/9 GC probe after solid-phase synthesis and cleavage



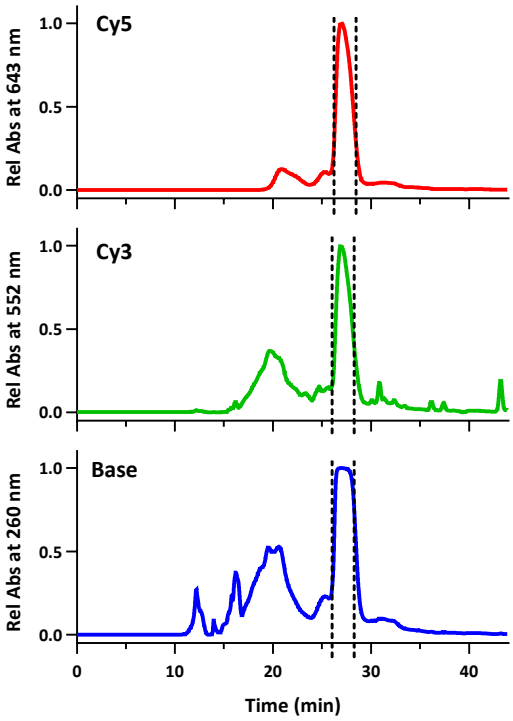
Separation was performed on C-18 column under a gradient of increasing acetonitrile in 0.5 M TEAA, with the product eluting at roughly 28 min.

Figure S2. AX-HPLC purification of 22/9/9 GC probe after conjugation to $(D\text{-}Arg)_9$ cell-penetrating peptide



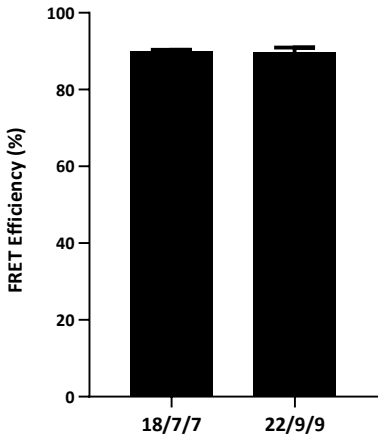
Separation was performed on Source 15q ion-exchange column under a gradient of increasing $NaClO_4$ in 1:1 formamide:Tris-HCl buffer, with the product eluting at roughly 24 min.

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



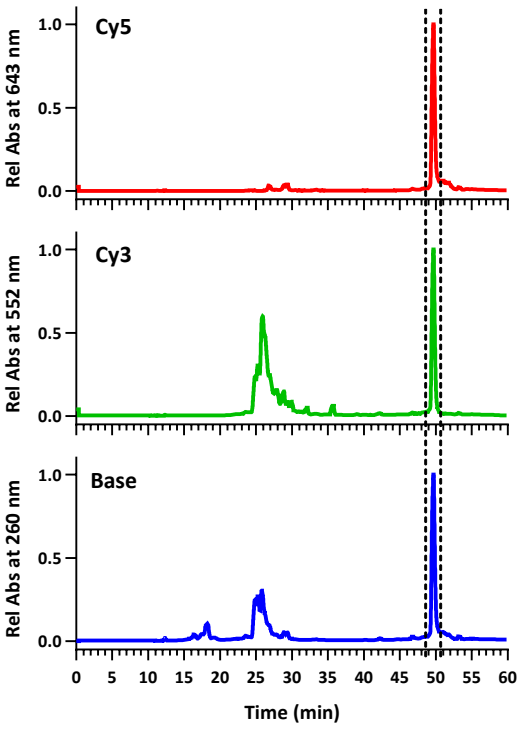
Separation was performed on C-18 column under a gradient of increasing acetonitrile in 0.5 M TEAA, with the product eluting at roughly 27 min.

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



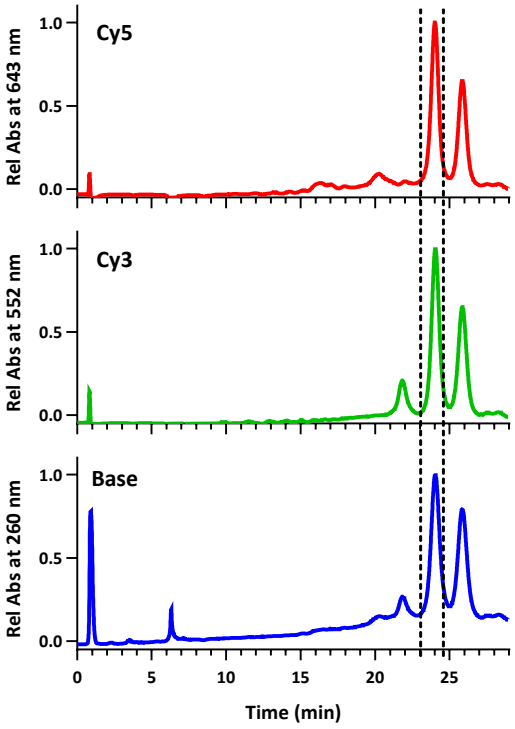
FRET efficiencies were measured for both probes at 1.0 μM in 1x STE buffer. 18/7/7 and 22/9/9 TIVA probes were synthesized according to [19].

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



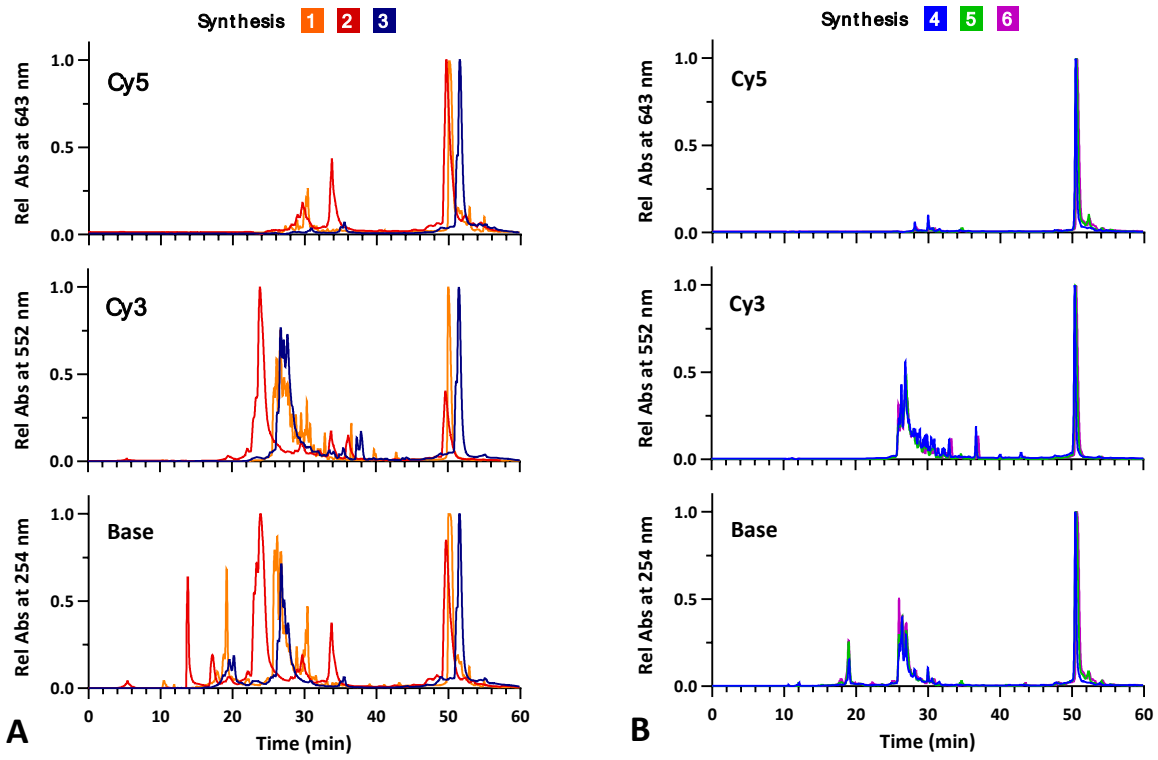
Separation was performed on C-18 column under a gradient of increasing acetonitrile in 0.5 M TEAA, with the product eluting at roughly 50 min.

Figure S6. AX-HPLC purification of 22/9/9 probe after conjugation to $(D\text{-}Arg)_9$ cell-penetrating peptide



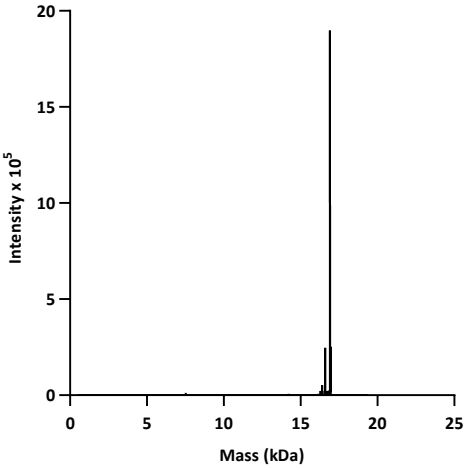
Separation was performed on Source 15q ion-exchange column under a gradient of increasing $NaClO_4$ in 1:1 formamide:Tris-HCl buffer, with the product eluting at roughly 24 min.

Figure 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 +$(D\text{-}Arg)_9$ TIVA



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