Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

An LC-MS chemical derivatization method for the measurement of five different one-carbon states of cellular tetrahydrofolate

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Fig. S1

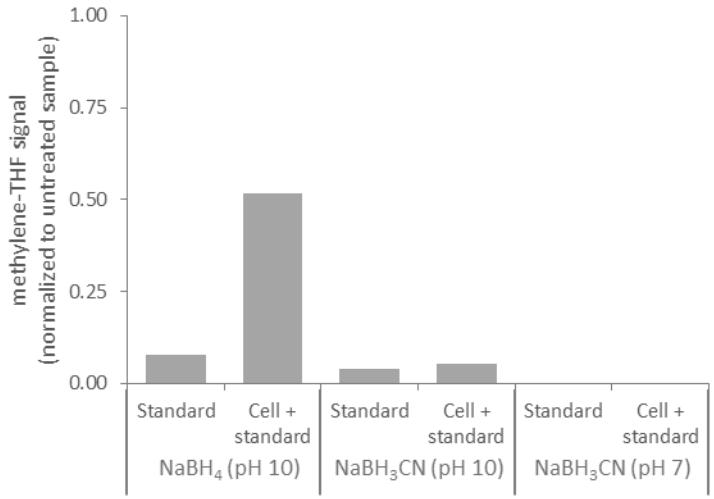

Fig. S1 Effectiveness of methylene-THF reduction with $NaBH_4$ (pH=10) or $NaBH_3CN$ (pH=7). The reduction reaction was carried out on either 1µg/mL methylene-THF standard or the same amount of standard spiked into HEK293T cell extracts with 25mM reducing agent. $NaBH_4$ was not tested at pH 7 due to its instability at neutral pH. Signals were normalized in each condition to the signal without the addition of reducing agent

Fig. S2

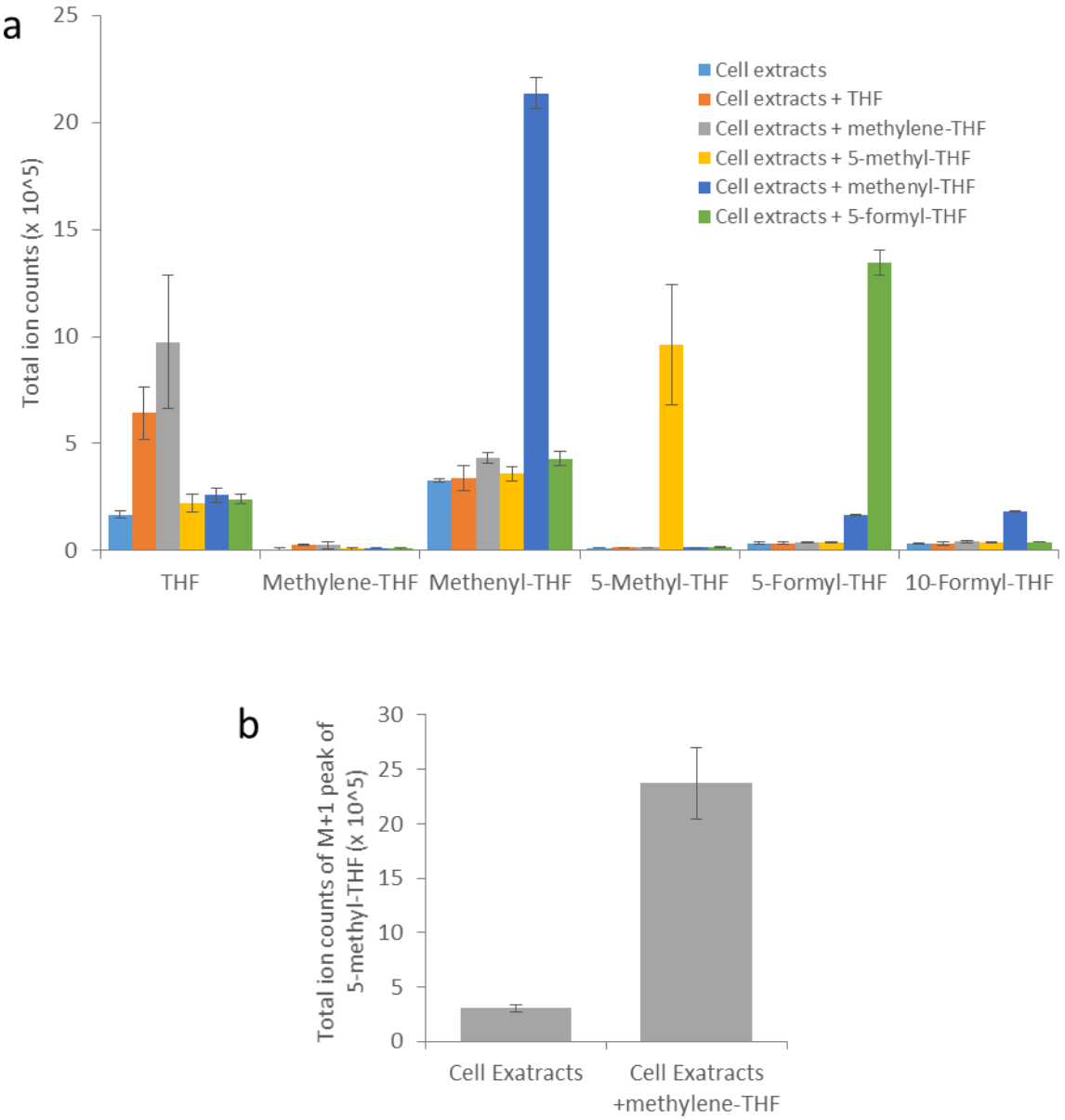

Fig. S2 Quantification of the absolute concentrations of THF species in growing HEK293T cells by spiking commercially available standards into cell lysates

(a) Total ion counts of THF species in HEK293T cell extracts with indicated spiked in standards. 4 pmol THF, methylene-THF, methenyl-THF, 5-formyl-THF and 5-methyl-THF were spiked into cell extracts before 5 min of 60°C incubation to abolish residual enzymatic activity converting 5-formyl-THF to methenyl-THF. Note that THF and methylene-THF are interconverted under such condition (Mean ± SD, N = 3).

(b) Absolute quantification of methylene-THF in HEK293T cells by $NaBD_3CN$ reduction method. 4 pmol methylene-THF was spiked into cell extracts containing 25mM $NaBD_3CN$. Note that $NaBD_3CN$ converted both intracellular and spiked-in methylene-THF to M+1 5-methyl-THF. Absolute values were obtained from comparing the difference in signal between the spiked samples and non-spiked samples (Mean ± SD, N = 3)