

Supplementary Materials: Activation of Tenofovir Alafenamide and Sofosbuvir in the Human Lung and Its Implications in the Development of Nucleoside/Nucleotide Prodrugs for Treating SARS-CoV-2 Pulmonary Infection

Jiapeng Li, Shuhan Liu, Jian Shi and Hao-Jie Zhu

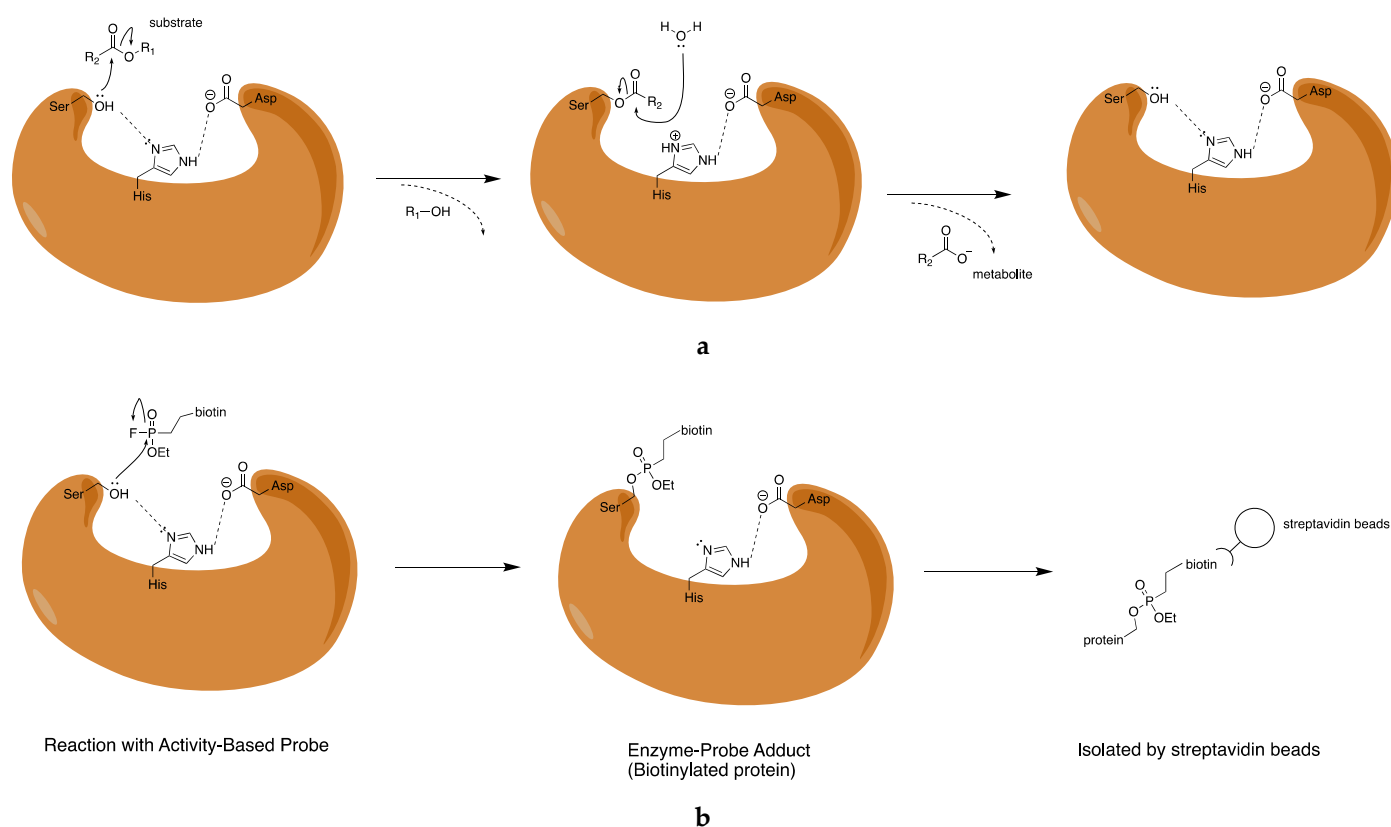


Figure S1. Reaction of serine hydrolases with an ester substrate (a) and biotin activity-based probe (b) via nucleophilic attacking [1].

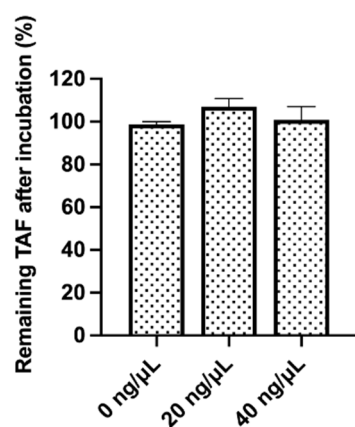


Figure S2. rhLYPLA1 showed no activity for TAF hydrolysis. 20 μM TAF was incubated with 20 and 40 ng/μL rhLYPLA1 in Tris pH 7.5 buffer at 37°C for 30 min. No significant reduction of TAF after incubations was identified.

Table S1. Demographic information of the tissue S9 donors.

Organ	Total sample number	Age (year)	Sex/ethnicity (number of samples)
Lung	4	22, 37, 49, 75	M/C (1); M/H (1); F/C (1); F/AA (1).
Liver	50	11-85	M/C (26); M/H (3); M/AA (1); F/C (19); F/A (1).

M = male; F = female. C = caucasian; H = hispanic; A = Asian; AA = African American.

Table S2. The gradient conditions of LC-MS/MS analysis.

Time (min)	A (%)	B (%)
0	97	3
68	70	30
73	60	40
75	20	80
78	20	80
79	97	3
90	end	end

Mobile phase: A: water containing 0.1% formic acid; B: acetonitrile containing 0.1% formic acid.

Table 3. Meta-analysis of the protein abundance of HINT1 in the human lung and liver.

Enzyme resource	Sample size	HINT1 Abundance ^a	Study reference
Pooled HLungS9	4 donors, triplicate measurement	0.165 ± 0.009 µg/mg total protein	[2]
Pooled HLS9	200 donors, triplicate measurement	0.588 ± 0.010 µg/mg total protein	
HLung S9	n = 3	2.4 ± 1.2 µg/mg total protein	[3]
HLS9	n = 3	4.5 ± 1.7 µg/mg total protein	
Primary human NHBE cells	n = 3	1.0 ± 0.3 µg/mg total protein	[4]
Primary human TBEC cells	n = 4	0.802 ± 0.039 µg/mg total protein	
Primary human hepatocytes	n = 7	0.50 ± 0.10 µg/mg total protein	[5]
Lung tissue autopsy from COVID-19 patients	n = 30	1.011 ± 0.721	[6]
Liver tissue autopsy from COVID-19 patients	n = 24	1.412 ± 0.328	
A549 cell line (lung cancer)	n = 3	0.919	[7]
Huh-7 cell line (liver cancer)	n = 3	1.14	

a: The unit (µg/mg total protein) is only applicable to absolute quantification data, is not applicable to the relative data obtained from the studies by Nie X. *et al.* [6] and Nusinow, D. P. *et al.* [7].

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