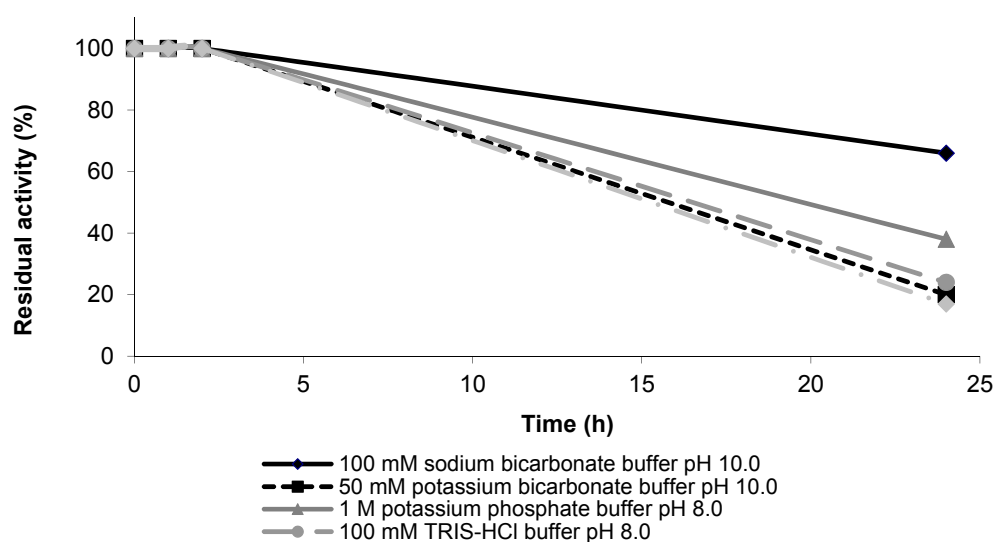


# Supplementary Materials: Immobilization of Neutral Protease from *Bacillus subtilis* for Regioselective Hydrolysis of Acetylated Nucleosides: Application to Capecitabine Synthesis

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**Figure S1.** Stability of native protease N in experimental conditions of immobilization.

(a)

Sequence of *B. subtilis* neutral protease A

AAATGSGTTLKGATVPLNISYEGGKYVLRDLSKPTGTQIITYDLQNRQSRLPGTLVSSTTKTFTSS  
 QRAAVDAHYNLGKVYDYFYNSFKRNSYDNKGSKIVSSVHYGTQYNN  
 AAWTGDQMIYGDGDSFFSPLSGSLDVT AHEMTHGVTQETANLIYENQPGALNESFSDVFGYFN  
 DTEDWDIGEDITVSQPALRSLSNPTKYNQPDNYANYRNLNPTDEGDYGGVHTNSGIPNKAAYN  
 TITKLGVSQSQQIYYRALTTYLTSPSTFKDAKAAALIQSARDLYGSTDAAKVEAAWNAVGL

Sequence of *S. aureus* metalloproteinase

AAATGTGKGVLDGTDKININSIDGGFSLDLTHQGKLSAYNFNDQTGQATLITNEDENFVKDDQ  
 RAGVDANYYAKQTYDYKNTFGRESYDNHGSPIVSLTHVNHYGGQDNRRNNAAWIGDKMIYGD  
 GDGRFTNLSGANDVVAHEITHGVTQQTANLEYKDQSGALNESFSDVFGYFVDDDFLMGEDV  
 YTPGKEGDALRSMSNPEQFGQPSHMKDYVYTEKDNNGVHTNSGIPNKAAYNVIQAIGKSKSEQI  
 YYRALTEYLTSNSNFKDLKDALYQAAKDLYEQQTAEQVYEAWNEVGVE

(b)

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Query 1  AAATGSGTTLKGATVPLNISIYEGGKYVLRDLKSKPTGTQIITYDLQNRQSRLPGTLVSSSTT 60
Sbjct 1  AAATGTGKGVLDGTDKIDINISIDGGFSLLEDLTHQGKLSAYNFNDQTGQA---TLITNED 56

Query 61  KTFTSSSQRAAVDAHYNLGKVYDYFYSNFKRNSYDNKGSKIVSSVH---YGTQYNN---A 114
Sbjct 57  ENFVKDDQQRAGVDANYAKQTYDYKNTFGRESYDNHGSPIVSLTHVNHYGGQDNRRNAA 116

Query 115  WTGDQMIYGDGDSFFSPLSGSLDVTAEHMGVTVQETANLIYENQPGALNESFSDVFGY 174
Sbjct 117  WIGDKMIYGDGGRFTNLGANDVVAHEITHGVTQQTANLEYKDQSGALNESFSDVFGY 176

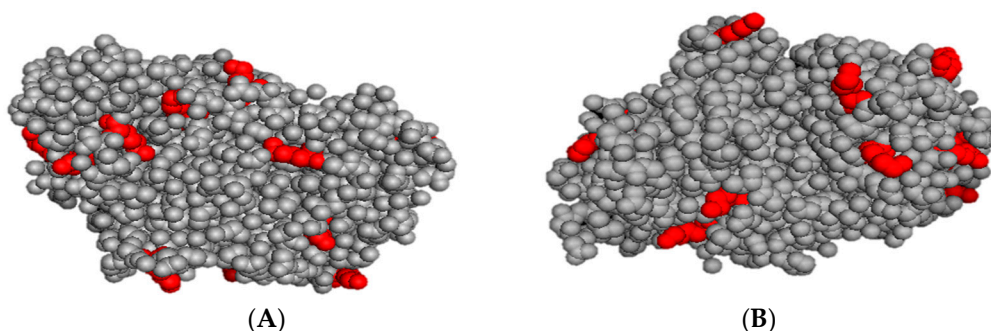
Query 175  FNDTEDWDIGEDITV---SQPALRSLSNPTKYNQPDNYANYRNLPTDEGDYGGVHTNSG 231
Sbjct 177  FVDEDFLMGEDVYTPGKEGDLRSMNSPEQFGQPSHMKDYVYT---EKDNGGVHTNSG 232

Query 232  IPNKAAYNTITKLGVSQSQQIYYRALTTYLTPSSTFKDAKAALIQSARDLYGSTDAAKVE 291
Sbjct 233  IPNKAAYNVIQAIGKSKSEQIYYRALTEYLTSNSNFKDLKDALYQAAKDLYEQQTAEQVY 292

Query 292  AAWNAVGL 299
          AWN VG+
Sbjct 293  EAWNEGV 300

```

**Figure S2.** (a) Sequences of *B. subtilis* neutral protease A (Protease N) and *S. aureus* metalloproteinase, (b) Blast alignment between *B. subtilis* neutral protease A and *S. aureus* metalloproteinase sequences.



**Figure S3.** Front (panel A) and back side (panel B) in the 3D structure of *S. aureus* metalloproteinase (pdb: 1qbq). Surface lysines are coloured in red.

### Analytical Characterization of Compounds 1a and 5a

**2,3-Di-O-Acetylluridine (1a):** TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 9:1): *R<sub>f</sub>* = 0.51. <sup>1</sup>H-NMR (400 MHz, [D<sub>6</sub>]DMSO, 25 °C): δ = 11.40 (s, 1 H, 3-NH), 7.89 (d, 1 H, 5-H), 6.00 (d, 1 H, 1-H), 5.70 (d, 1 H, 6-H), 5.50 (s, 1 H, OH in 5), 5.30 (m, 2 H, 2-H, 3-H), 4.14 (m, 1 H, 4-H) 3.64 (m, 2 H, 5-H) 2.10–2.02 (s, 6 H, 2 OAc) ppm. MS: calcd. for [M + 1]<sup>+</sup>: 351.26; found 351.00.

**2,3-Di-O-Acetylcytidine (5a):** TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 9:1): *R<sub>f</sub>* = 0.50. <sup>1</sup>H-NMR (400 MHz, [D<sub>6</sub>]DMSO, 25 °C): δ = 7.80 (d, 1 H, 5-H), 7.30 (s, 2 H, NH<sub>2</sub>), 6.00 (d, 1 H, 1-H), 5.70 (d, 1 H, 6-H), 5.40 (m, 1 H, 2-H, 3-H, OH in 5), 4.10 (m, 1 H, 4-H), 3.70 (m, 2 H, 5-H), 2.10–2.00 (s, 6 H, 2 OAc) ppm. MS: calcd. for [M + Na]<sup>+</sup>: 350.28; found 350.10.