|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **TRMT1 Co-Incubation** | ***kobs***  **(hr-1)** | ***kobs***  **+/-** |  | ***KD***  **(µM)** | ***KD***  **+/-** |
| Mpro WT | n.d. | n.d. |  | 4.76 | 1.08 |
| Mpro C145A | 1.97 | 0.07 |  | 0.86 | 0.03 |
| No Protease | 2.10 | 0.05 |  | 0.80 | 0.05 |

**Figure 2–source data 1.** Table of methyltransferase activity (*k*obs) and tRNA binding affinity (*K*D) parameters for TRMT1 after an 18-hour incubation with Mpro WT, C145A, or no protease, corresponding to the fits of plots presented in **Figure 2C** and **2D**, respectively. TRMT1 tRNA modifying activity was measured by radiolabel-based methyltransferase assays with *S*-[methyl-14C]-adenosyl methionine and fit to a first-order exponential to obtain *k*obs (see also **Figure 2C**); TRMT1 incubated with Mpro WT did not have activity during the 4-hour time course, so *k*obs is listed as n.d. (not determined) for this condition. TRMT1-tRNA binding affinity was determined by EMSA experiments (see **Figure 2–figure supplement 1**) and fit to a standard single-site ligand binding equation to obtain *KD*s (see also **Figure 2D**). All kinetic and binding experiments were carried out in triplicate and errors are reported above as the standard error of the fits shown **Figure 2C** and **2D**.