The process of HDAC11 Assay Development: follow-up for time-course study

As a follow-up for the increased fluorescence signals at 0 min of the HDAC11 reaction for any given concentration of the protein from the previous dataset (Fig 1 and Fig 2, *dataset*), a test was performed to check that instead of the reaction producing this fluorescence at time 0 min, the protein might be contributing in this fluorescence.

Here, two sets of reactions were set-up in parallel, wherein 1 set contained both protein and the substrate, while the second set contained only the protein. The measurements were taken for increasing concentrations of HDAC11 at only 0 min. The reaction recipe is enlisted in Table 1. Samples containing no protein (only substrate) were included in the set-up to serve as blank in case of HDAC11+substrate. While in case of only HDAC11 (no substrate), assay buffer containing protein buffer (in place of protein) served as the blank. The results are plotted in Fig 1 (blank subtracted).

Table 1. Reaction recipe for measurements at time 0 min.

7.5 µl Reaction volume	
HDAC11 (µM)	0-2
Boc-Lys-(TFA)-AMC (µM)	200 or 0
Assay buffer	20 mM HEPES, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2 0.5% BSA (added freshly)
Reaction time at RT (25 ºC)	0 min
7.5 µl Developer	
Developer conc. (5X stock)	1X
Incubation time	30 min

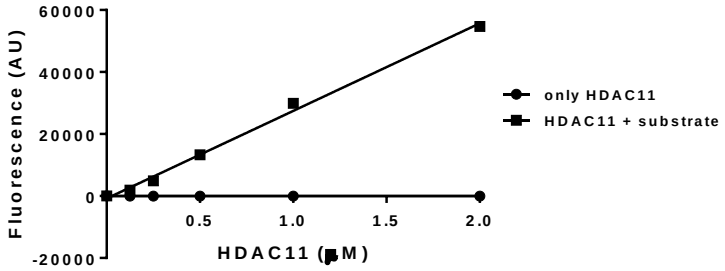

Fig 1. Time zero readings for HDAC11.

Observations from Fig 1:

1. For samples with only HDAC11 (no substrate added), there is no increase in fluorescence with increasing HDAC11 concentration.
2. For samples with both HDAC11 and substrate, the fluorescence increases.

Inferences from the above observations:

1. The protein alone does not contribute to any increase in fluorescence at time zero.
2. HDAC11 appears to be an enzyme exhibiting pre-steady state kinetics (burst phase).