

Impact of 40 Hz Data Acquisition on Kinetic Characterization on Octet® R8e

This study supports the impact of an enhanced data acquisition rate of 40 Hz on biolayer interferometry (BLI) kinetic analysis using the Octet® R8e, which can improve accuracy in data. It summarizes internal scientific analysis comparing 5 Hz and 40 Hz sampling on the same sensors and plate under identical conditions.

Assay Information

Carbonic anhydrase II (biotinylated) captured on Super Streptavidin (SSA) biosensors; Furosemide (330 Da) at 10 µM; PBS + 0.5% DMSO; 384 tilted-well plate; double reference subtraction; 1:1 model fit.

Results

Results below are derived from a comparative assay in which datasets were acquired at 5 Hz and 40 Hz using the same biosensors and sample plate. All data were double-reference-corrected and fitted to a 1:1 binding model. Assay fit metrics and comparison are summarized in Tables 1 and 2, respectively.

Table 1 - Comparative Fit Metrics

	K_D (nM)	K_D error	k_a ($M^{-1}s^{-1}$)	k_a error	k_d (s^{-1})	k_d error	R_{max}	R_{max} error
5 Hz	989	1.23 E-8	4.26E4	4.91E2	4.21E-2	1.978E-4	0.1311	0.0002
40 Hz	979	6.96 E-9	4.67E4	3.15E2	4.58E-2	1.16E-4	0.1086	0.0001

Table 2 - Parameter Uncertainty Reduction at 40 Hz (vs 5 Hz)

Parameter	Error 5 Hz	40 Hz	Reduction (%)
K_D error	1.23E-08	6.96E-09	43.4%
k_a error	4.91E+02	3.15E+02	35.8%
k_d error	1.98E-04	1.16E-04	41.4%
R_{max} error	2.00E-04	1.00E-04	50.0%

Discussion

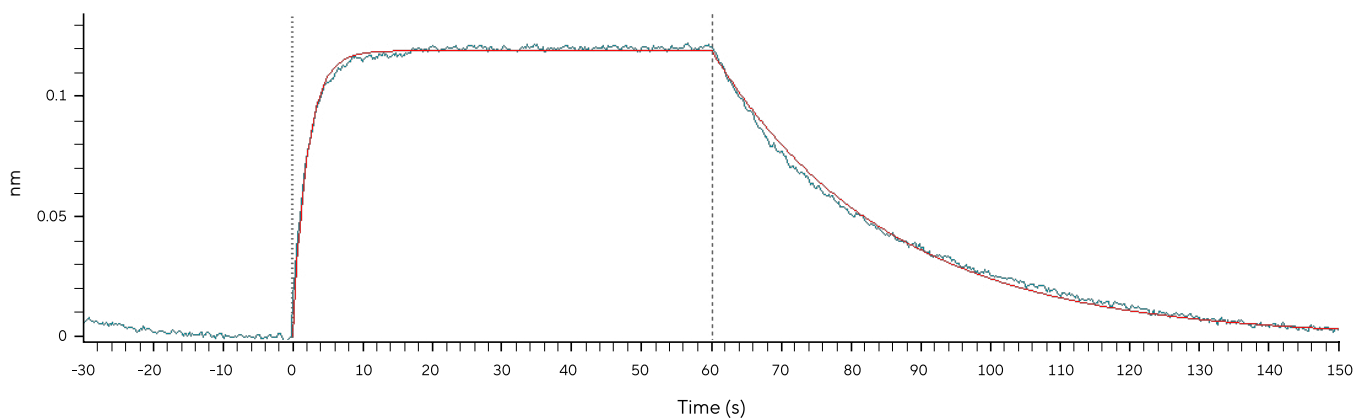
The affinity estimate remained consistent (989 nM at 5 Hz vs 979 nM at 40 Hz) (Table 1), indicating no systematic bias introduced by the higher sampling rate. Parameter uncertainties decreased materially at 40 Hz, K_D error (–43%), k_a error (–36%), k_d error (–41%), R_{max} error (–50%) – consistent with improved identifiability from denser sampling of rapid association and early dissociation phases (Table 2).

Conclusions

Increasing the acquisition rate from 5 Hz to 40 Hz preserved the estimated affinity ($K_D \approx 1 \mu\text{M}$ in both cases) while reducing parameter-fitting uncertainty (K_D , k_a , k_d , Rmax). The higher sampling frequency improved early resolution of association/dissociation, enabling a more accurate model fit and narrower parameter errors without introducing bias. The 40 Hz acquisition provides higher time resolution, capturing transient curvature that may be undersampled at 5 Hz. For fast on/off interactions, 40 Hz reduces the risk of biased estimates caused by sparse sampling of early time points. Improved precision, reflected in smaller errors, enhances reproducibility and confidence intervals reported to regulators and procurement bodies. The consistency of K_D across rates demonstrates that 40 Hz refines estimates without altering the underlying interaction.

Figures

A



B

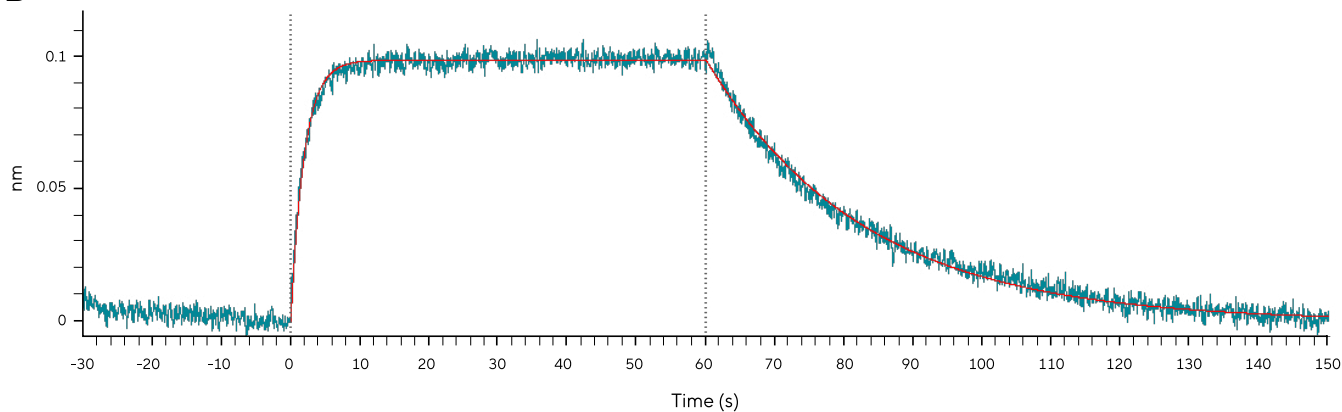


Figure 1: Kinetic analysis of the interaction between carbonic anhydrase II and a single concentration of Furosemide ($10 \mu\text{M}$) at 5 Hz (A) and 40 Hz (B) at 1000 rpm and 25°C . For all sensorgrams, Savitzky-Golay filtering was not used. Red line shows the 1:1 binding model fit to the collected data.

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