Specific Inhibition of a Designed Metallopeptide Catalyst by Organic-Inorganic Cooperativity

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Supporting Information

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General Considerations.

Solvents and reagents were purchased from Fisher Scientific and used as received. Millipore ultra-purified water (18 MΩ) was used in all cases.

Experimental protocols. The terms "aqueous buffer" and "10X aqueous buffer" refers to aqueous solutions of 0.1 M and 1M *t*-BuNHOH·HCl at the indicated pH, respectively. Stock solutions of substrate peptides and metallopeptide catalysts were prepared in water and frozen in between uses. All modification reactions were carried out in microcentrifuge tubes (600 µL capacity). Mixing was maintained at 25 °C using a rotary mixer.

Synthesis of known compounds. The dirhodium precursor *cis*-$Rh_2(tfa)_2(OAc)_2$,[i] substrates $E3_gX$ (X = W, F, H, M, C) and $E3_eW$,[ii] catalysts $K3_{a,e}Rh_2$ and $K3_{g,d}Rh_2$,[ii] and diazo reagent [2-(2-methoxyethoxy)ethoxy]ethyl (*E*)-4-phenyl-2-diazo-3-butenoate (**1**)[iiia] were prepared and purified according to published procedures.

Mass Spectrometry. MALDI-MS was performed on a Bruker Daltonics Autoflex MALDI-TOF/TOF mass spectrometer with CHCA matrix (10 mg/mL, Thermo Scientific Pierce). Data analysis was performed with the mMass program.[iv]

Circular Dichroism Spectroscopy. Thermal denaturation experiments (–5 – 105 °C with a gradient of 1 °C/min) were performed on a Jasco-J810 spectropolarimeter with a Peltier temperature controller (Jasco PTC423S). Solutions of 1:1 E3-peptide and K3-metallopeptide (both components 100 or 33 µM) in aqueous buffer in a 0.1 cm sealed cell were analyzed, and ellipticity data were acquired at 222 nm (red data points, S-5). When imidazole additive was utilized, ellipticity data were acquired at 225 nm. Temperature denaturation curves were fit to a two-state unfolding model and plotted (black line, S-5) as fraction unfolded vs. temperature as described previously by Lavigne et al.[v] Error associated with the non-linear least squared-determined T_m was determined using the freely available "Solver Statistics" macro for *Microsoft Excel*. Dissociation constants (K_d) were determined at 25 and 37 °C by averaging K_d values over a ±1 °C range. The error in K_d values was determined as the standard error between K_d^{obs} and K_d^{calc} over the 2 °C temperature range.

Experimental.

Representative procedure for inhibition of catalytic side-chain modification: Reaction of $E3_gW$ (25μM), diazo 1 (50 equiv), and $K3_{a,e}Rh_2$ (1 mol %) with $E3_gH$ (0.01–10μM) inhibitor. Bulk stock solutions of $E3_gW$ (2.5 mM), $K3_{a,e}Rh_2$ (25 μM), and $E3_gH$ (2.5 mM) were prepared. The inhibitor stock solution of $E3_gH$ was subsequently diluted to make four additional stock solutions of 0.25, 2.5, 25, and 250 μM. A stock solution of diazo reagent **1** (4.2 mg in 198 μL *t*-BuOH and 198 μL H_2O, ~31.4 mM) was also prepared. To a microcentrifuge tube a reaction stock solution was prepared by transferring $E3_gW$ bulk stock (25 μL, 62.5 μM final concn) and $K3_{a,e}Rh_2$ bulk stock (25 μL, 0.63 μM final concn) was dissolved in water (850 μL) and 10X aqueous buffer (100 μL, pH 6.2). This reaction stock solution was transferred in aliquots (10 μL) to separate microcentrifuge tubes and the requisite volume of $E3_gH$ inhibitor and water were added to bring the reaction volume to 25 μL. The reaction was initiated by addition of diazo stock (2 μL, 1.25 mM final concn). The total reaction volume was 25 μL with 2% *t*-BuOH co-solvent. The reaction tube was initially mixed for ca. 30 s with a bench-top vortex mixer and was then placed on a rotary mixer to react at room temperature. The reaction was quenched after 20–50 min by addition of 75 μL of 70% MeCN in H_2O with 0.1% TFA. An aliquot of this solution (0.5 μL, ~3 pmol $E3_gW$) was spotted for MALDI-TOF analysis of modification initial rate.

Modification Analysis: E3W peptide conversion was determined exclusively from the ratio of modified to unmodified peptide using peak intensity from MALDI-TOF MS analysis and is uncorrected. Kinetic inhibition reactions were typically run in duplicate. Three spectra from different locations on the sample spot were acquired and averaged over both reactions (6 total measurements) to obtain the reported conversion. This analysis was validated in our previous study in which conversion of $E3_gW$ was monitored by reverse-phase HPLC/UV absorbance spectroscopy and MALDI-TOF MS methods as a function of time, which yielded comparable conversion data with variations ≤10%.[ii] We also demonstrate here that initial rates data derived from MS analysis for the modification of substrate $E3_gW$ at varying catalyst concentration yields a linear correlation, implying a first-order dependence on [$K3_{a,e}Rh_2$].

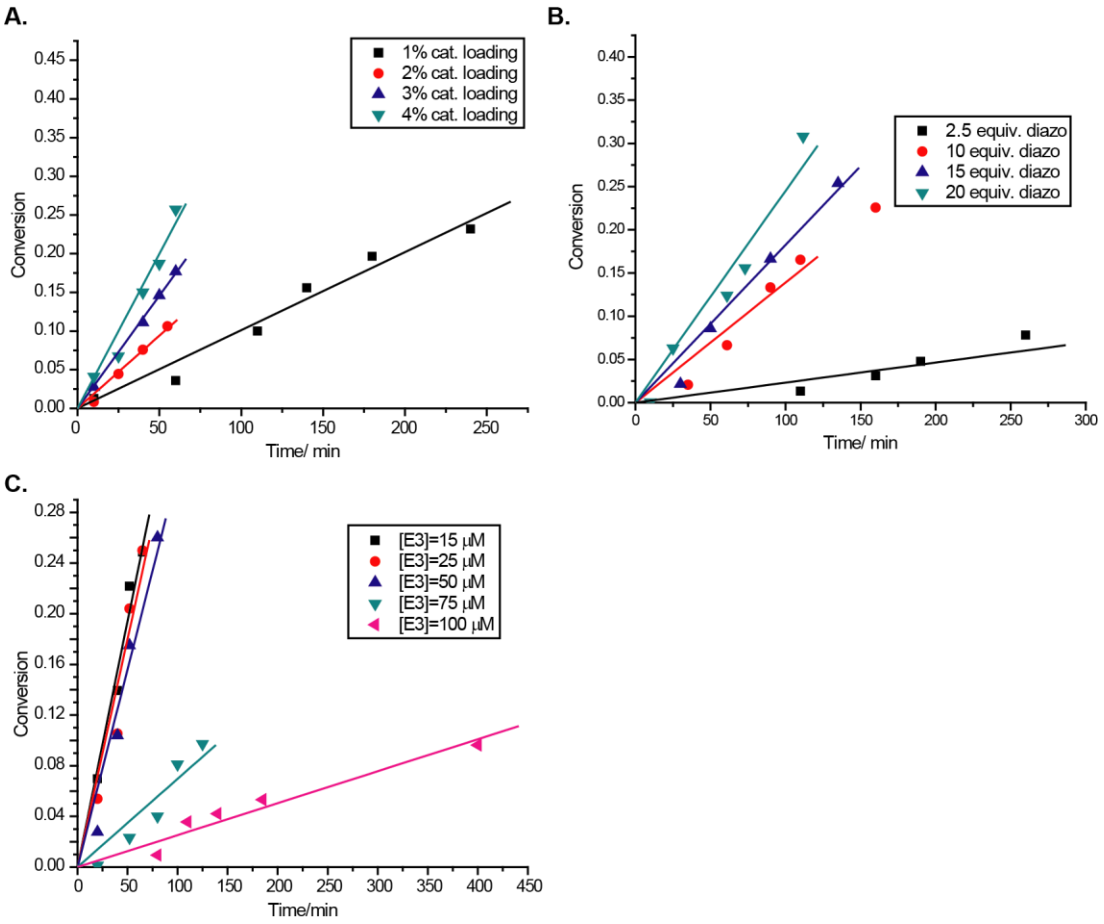

Figure S-1. Concentration dependence for $E3_gW$ conversion on metallopeptide (A), diazo (B) and substrate (C). Conditions: A) $E3_gW$ (25 μM), K3-Rh_2 (0.25–1.0 μM), and diazo **1** (375 μM) in aq buffer. B) $E3_gW$ (25 μM), K3-Rh_2 (0.50 μM), and diazo **1** (67.5-500 μM) in aq buffer. C) $E3_gW$ (15-100 μM), K3-Rh_2 (0.50 μM), and diazo **1** (750 μM) in aq buffer. The modification ratio was determined by MALDI–TOF MS and the initial rate was calculated using linear fitting at low conversion and then plotted as Figure 2.

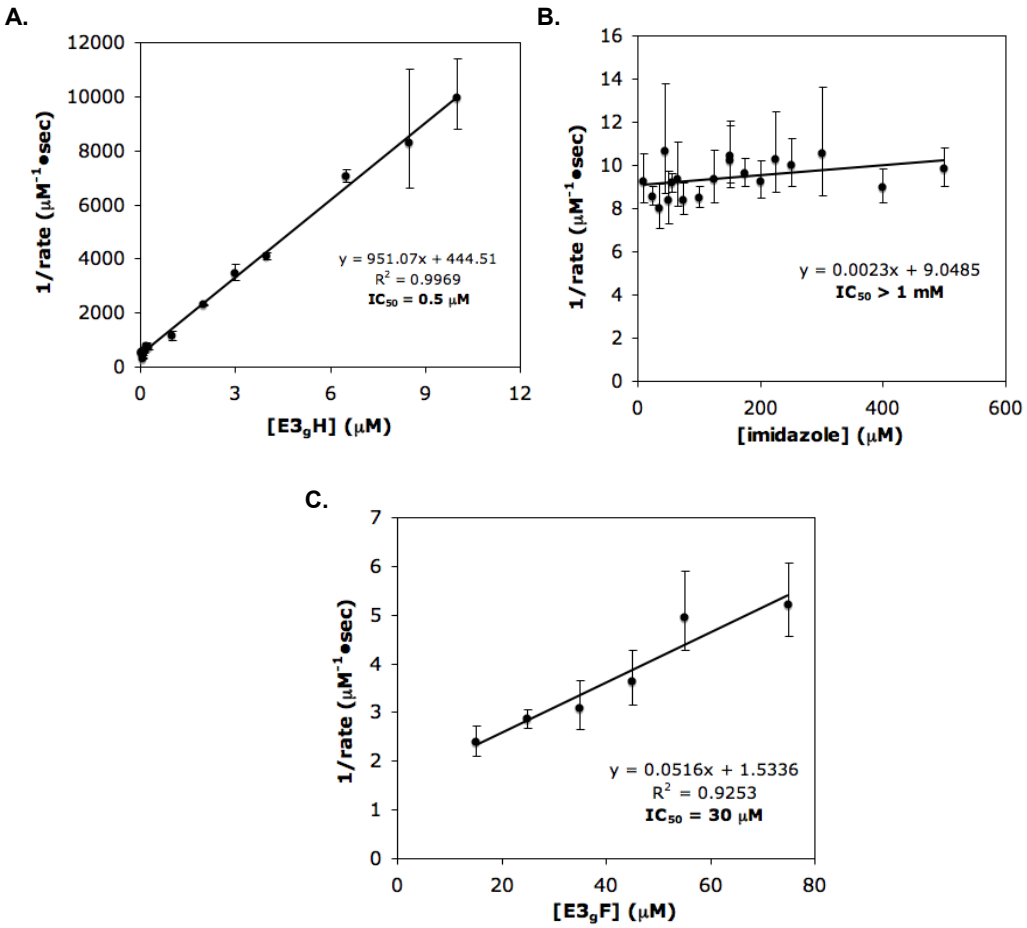

Figure S-2. Dixon plots (not illustrated in Figure 3). A) $E3_gW$ (5 μM)–$K3_{a,e}Rh_2$ (50 nM) with $E3_gH$ (0.025–10 μM) inhibitor. B) $E3_gW$ (25 μM)–$K3_{a,e}Rh_2$ (250 nM) with imidazole (10–500 μM) inhibitor. C) $E3_eW$ (25 μM)–$K3_{g,d}Rh_2$ (250 nM) with $E3_gF$ (15–75 μM) inhibitor. See above for representative procedure.

Thermal Denaturation Profiles:

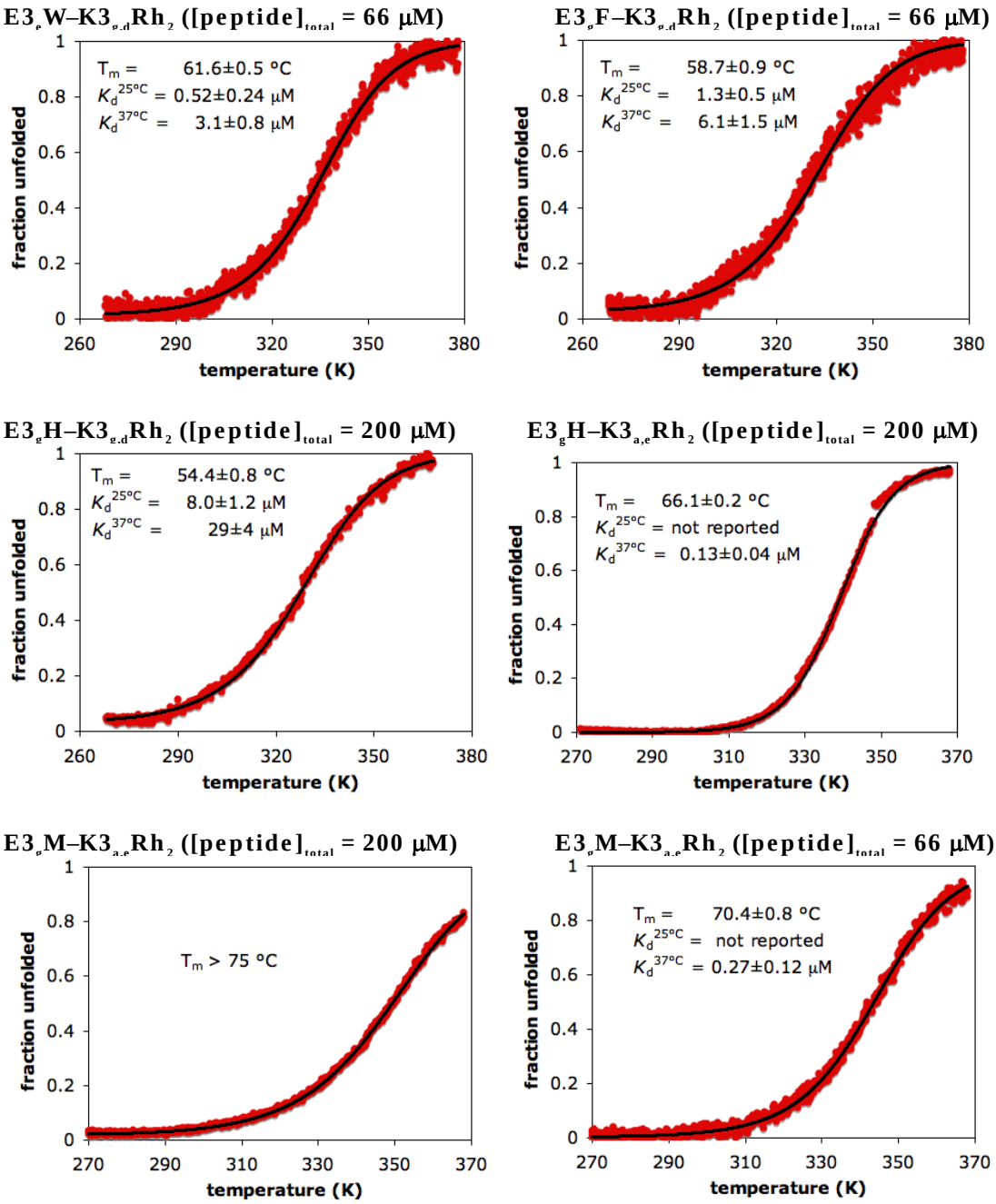

Figure S-3. Thermal denaturation profiles with observed (red) and fit (black) data. Apparent dissociation constants (K_d) values were determined from fits of fraction unfolded values at the indicated temperatures. Data for $E3_gM–K3_{a,e}Rh_2$ and $E3_gH–K3_{a,e}Rh_2$ is reproduced from our previous work.[vi]

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