Table 1: Comparison of Kinetic Parameters and Activation Barriers for Hydrazone Formation and Friedel–Crafts Reactions

Reaction	**System/ Catalyst**	k_{cat} **(s^{-1} $\times 10^{-2}$)**	K_M **(mM)**	k_{cat}/K_M **(M^{-1} s^{-1})**	$\Delta G^{\ddagger}$**, QM (kcal/mol)**	$\Delta G^{\ddagger}$**, exp (kcal/mol)**	**Key Mechanistic Steps**	**Reference**
Hydrazone formation	Buffer only	n.a.	n.a.	$\sim$0.001	23.0 (uncatalyzed)	—	1. Hydrazine attacks carbonyl 2. Hemi-aminal formation 3. Dehydration to hydrazone	Drienovská et al., *Nat Chem* 2018
Hydrazone formation	LmrR (no pAF)	n.a.	n.a.	$\sim$0.002	—	—	1. Hydrazine attacks carbonyl 2. Hemi-aminal formation 3. Dehydration to hydrazone	Drienovská et al., *Nat Chem* 2018
Hydrazone formation	LmrR_pAF	1.2	0.017	0.72	15.2 (hemiaminal, S) 11.0 (hemiaminal, R) 8.7 (dehydration, S) 19.4 (dehydration, R)	—	1. pAF forms iminium (Schiff base) with carbonyl 2. Hydrazine attacks iminium 3. Dehydration to hydrazone	Drienovská et al., *Nat Chem* 2018
Friedel–Crafts alkylation	Buffer only	n.a.	n.a.	0.01	—	24.7	1. Indole attacks enal (direct nucleophilic addition) 2. Proton transfer 3. Product release	Leveson-Gower et al., *ACS Catal* 2021
Friedel–Crafts alkylation	LmrR (no pAF)	n.a.	n.a.	0.02	—	23.4	1. Indole attacks enal (direct nucleophilic addition) 2. Proton transfer 3. Product release	Leveson-Gower et al., *ACS Catal* 2021
Friedel–Crafts alkylation	LmrR_pAF	1.45	18.2	0.80	—	20.8	1. pAF forms iminium (Schiff base) with enal 2. Indole attacks iminium (conjugate addition) 3. Proton transfer 4. Hydrolysis releases product and regenerates catalyst	Leveson-Gower et al., *ACS Catal* 2021

How to Interpret the Table

Hydrazone formation

- **Buffer only (no protein):** The reaction is extremely slow. Only a theoretical (QM) barrier is available for this uncatalyzed reaction in water ($\Delta G^{\ddagger}_{\text{QM}} \approx 23$ kcal/mol); the experimental barrier cannot be reliably measured due to the low rate.
- **LmrR (no pAF):** The wild-type protein gives only a marginal rate enhancement over buffer, with no measurable experimental or QM barrier specific to the protein system. The mechanism is the same as in buffer.
- **LmrR_pAF:** Incorporation of pAF provides a dramatic rate enhancement. Only QM barriers are available for this artificial enzyme ($\Delta G^{\ddagger}_{\text{QM}}$ as low as 8.7–15.2 kcal/mol for key steps), reflecting the new iminium-based mechanism.

Friedel–Crafts alkylation

- **Buffer only (no protein):** The reaction is slow, with a measurable experimental barrier ($\Delta G^{\ddagger}_{\text{exp}} \approx 24.7$ kcal/mol).
- **LmrR (no pAF):** The wild-type protein provides a slight rate enhancement ($\Delta G^{\ddagger}_{\text{exp}} \approx 23.4$ kcal/mol), but no significant catalysis or mechanistic change.
- **LmrR_pAF:** The artificial enzyme lowers the experimental barrier substantially ($\Delta G^{\ddagger}_{\text{exp}} = 20.8$ kcal/mol), demonstrating true catalytic activity via an iminium mechanism.

Significance

- **Catalysis lowers the activation barrier** ($\Delta G^{\ddagger}$), leading to increased reaction rates.
- **Agreement between QM and experimental barriers** in buffer/water validates the mechanistic understanding of the uncatalyzed process.
- **Artificial enzymes like LmrR_pAF** can dramatically accelerate reactions by introducing new catalytic mechanisms (e.g., iminium catalysis for hydrazone formation).