

Overexpression and Inactivation of MMP-9: A Novel Chronic Wound Remedy

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Background

Chronic wounds or non-healing wounds take longer than 3 months to heal. They severely negatively impact the quality of life of those affected with pain, reduced mobility, odor, and persistent infection. 1-2% of the global population is affected by chronic wounds, costing >\$150 billion annually.^{1,2}

Elevated levels of matrix metalloproteinase-9 (MMP-9), a zinc protease necessary for wound healing, is a major contributor to non-healing wounds. We have shown that hypochlorous acid (HOCl), a natural product produced as part of the innate defense system, acts both as an effective antimicrobial in a chronic wound model and an odor control agent (**Figure 1**). We hypothesize that HOCl will also inactivate MMP-9 and promote wound healing. Here, we developed a method to overexpress, purify, and test the activity of the active form of MMP-9 for future testing with HOCl.

HOCl: Odor and antimicrobial activity

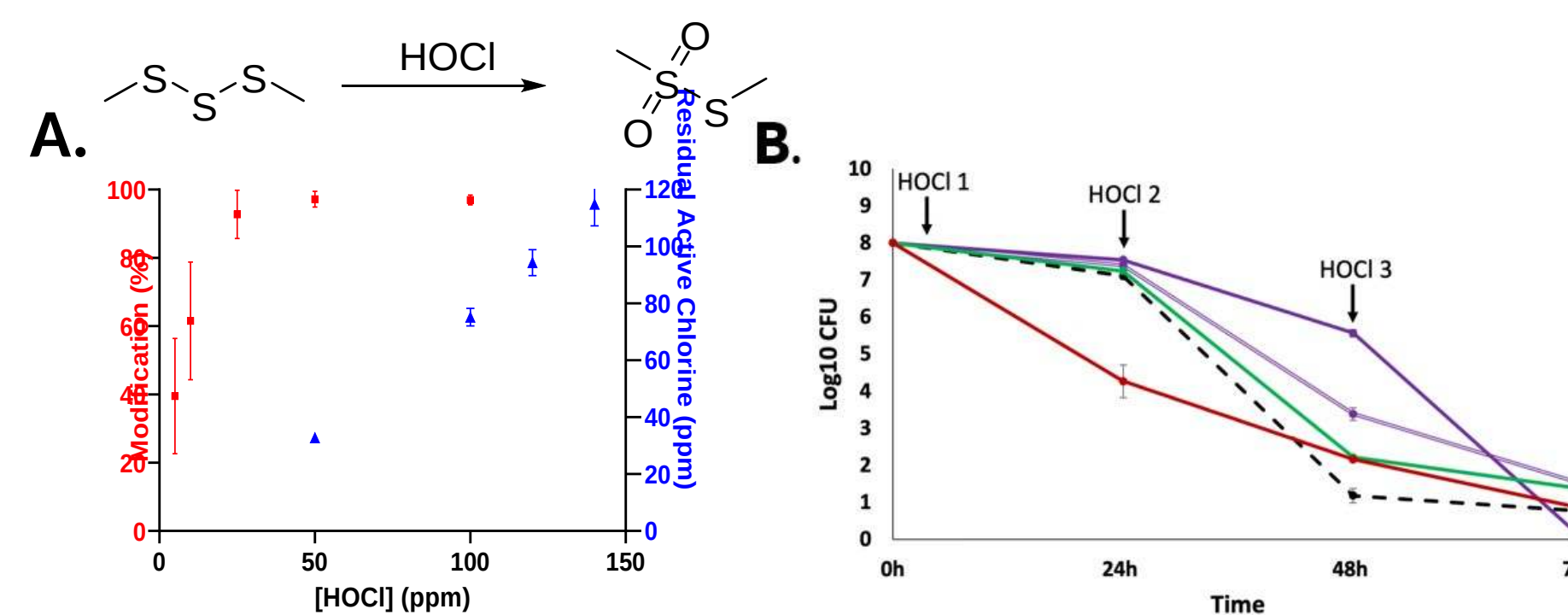
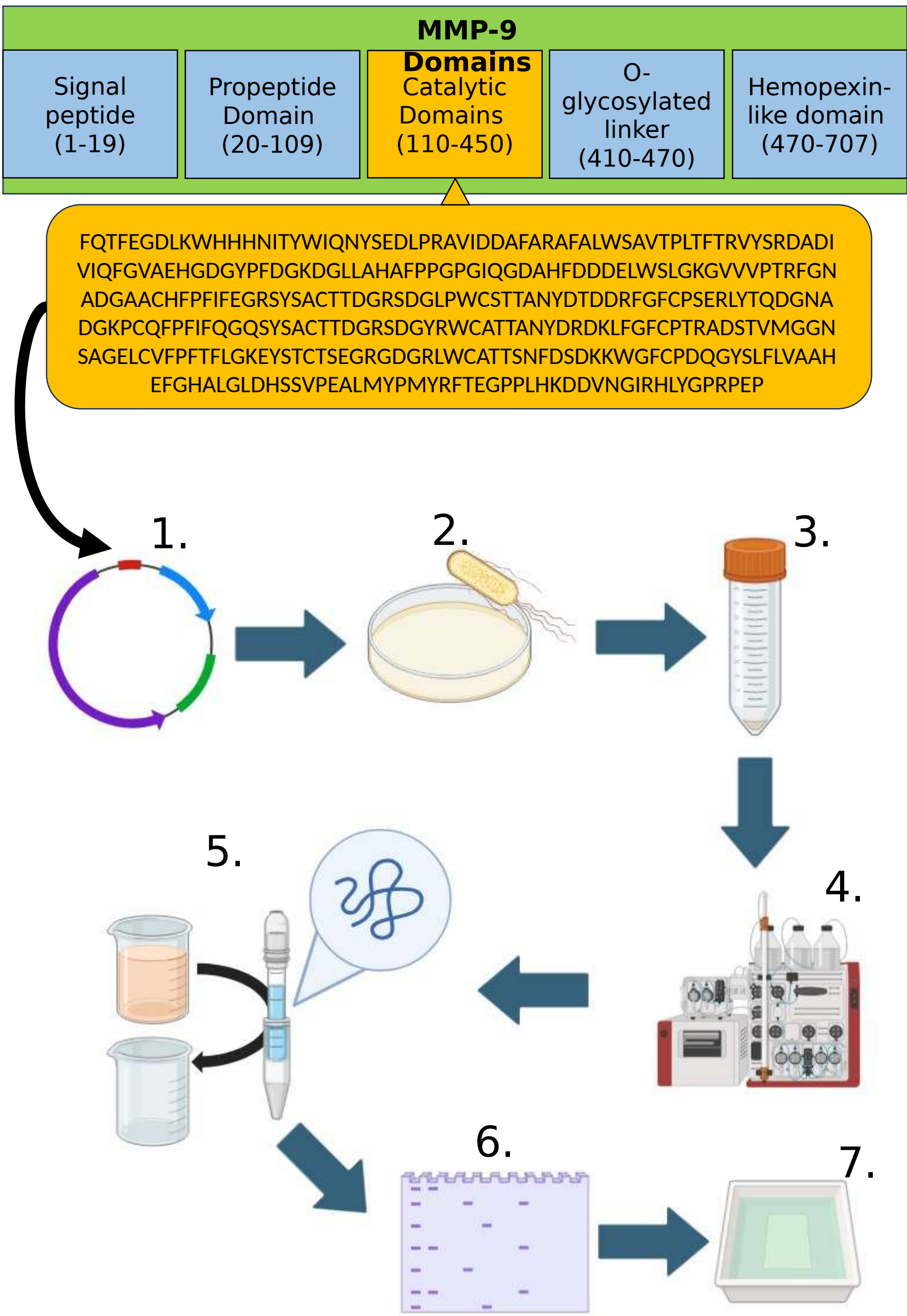


Figure 1: Antimicrobial and odor control properties of HOCl. **A.** Modification of dimethyl trisulfide (DMTS) by HOCl detected by HPLC.³ **B.** Efficacy of HOCl gel against chronic wound biofilms. Multiple doses of HOCl gel applied at 5h, 24h and 48h to biofilm containing five-species of bacteria: *Staphylococcus aureus*; *Streptococcus pyogenes*; *Enterococcus faecalis*; *Escherichia coli*; and *P. aeruginosa*.⁴

Methods



Purification

1. Transformation of pET 21+/MMP-9 into BL21*DE3 *E. coli* cells
2. Cells were plated on agar with 50 μ g/mL ampicillin
3. Grow cells in Lennox broth and induce with IPTG
4. Lyse cells then purify protein with Ni²⁺ affinity chromatography
5. Buffer exchange to refolding buffer containing Ca²⁺ and Zn²⁺
6. SDS-PAGE with 0.05% gelatin
7. Develop with Ca²⁺ and Zn²⁺ and stain with Coomassie Blue dye

Purification of MMP-9

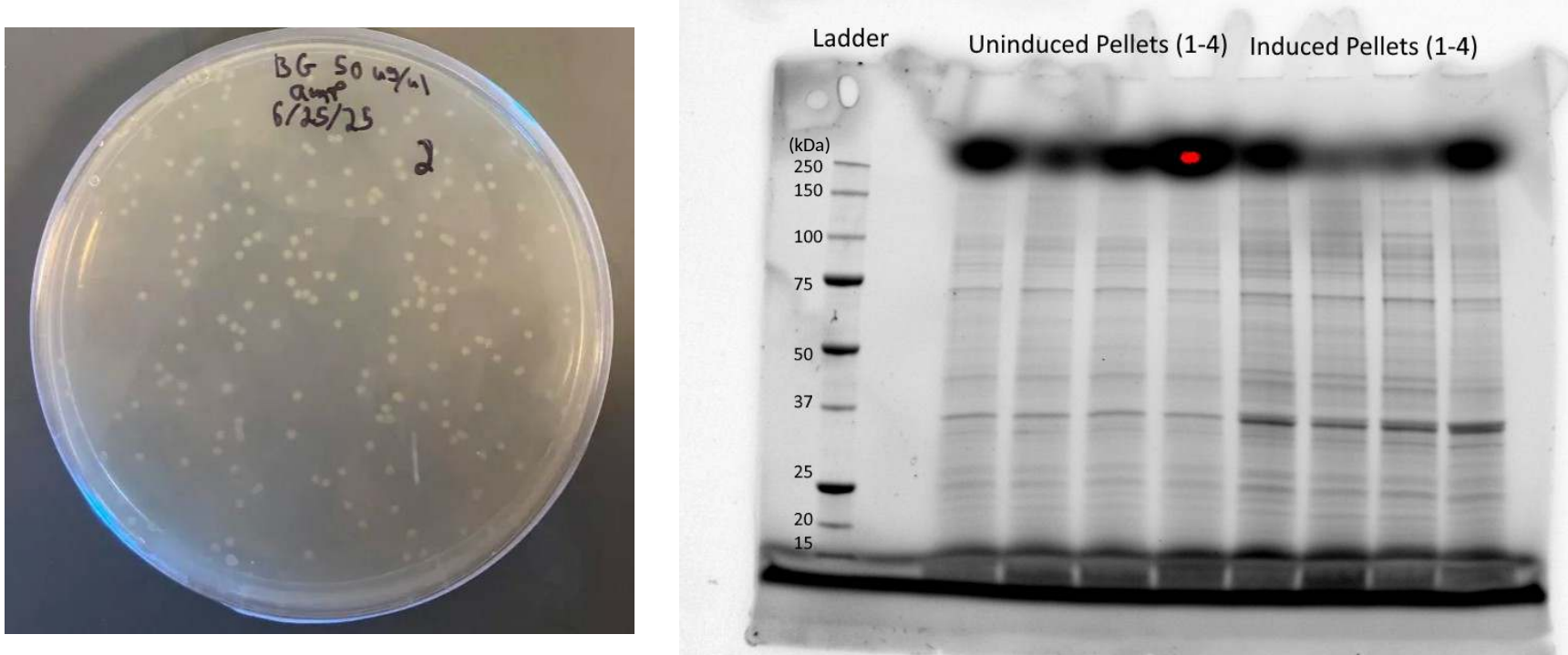


Figure 2: SDS-PAGE analysis of uninduced and induced *E. coli* pellets.

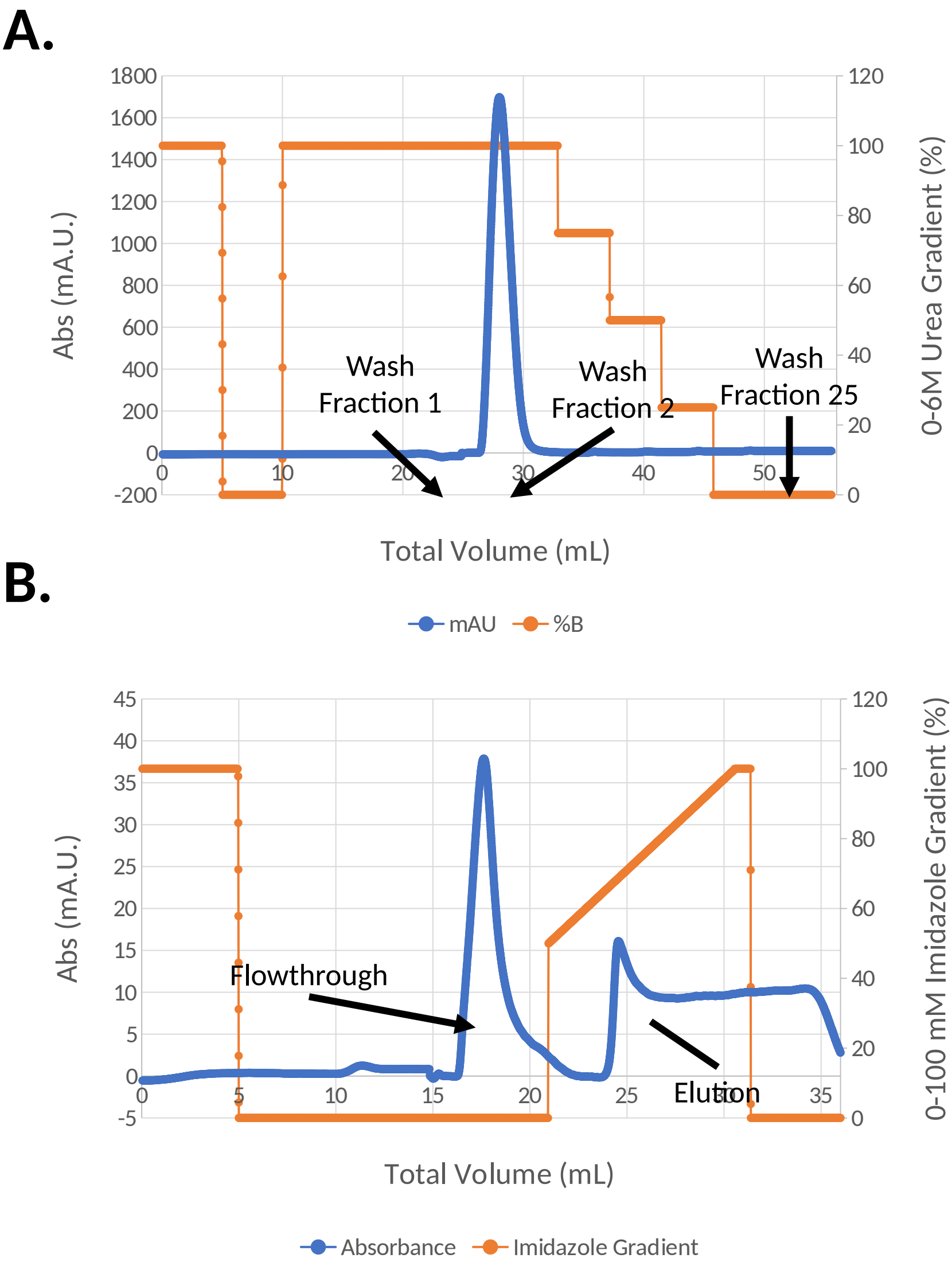


Figure 3: AKTA affinity chromatography purification with a Ni²⁺ column. **A.** Loading the lysate and washing with urea and **B.** Elution of MMP-9 with imidazole.

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Verifying MMP-9 Purification and Activity

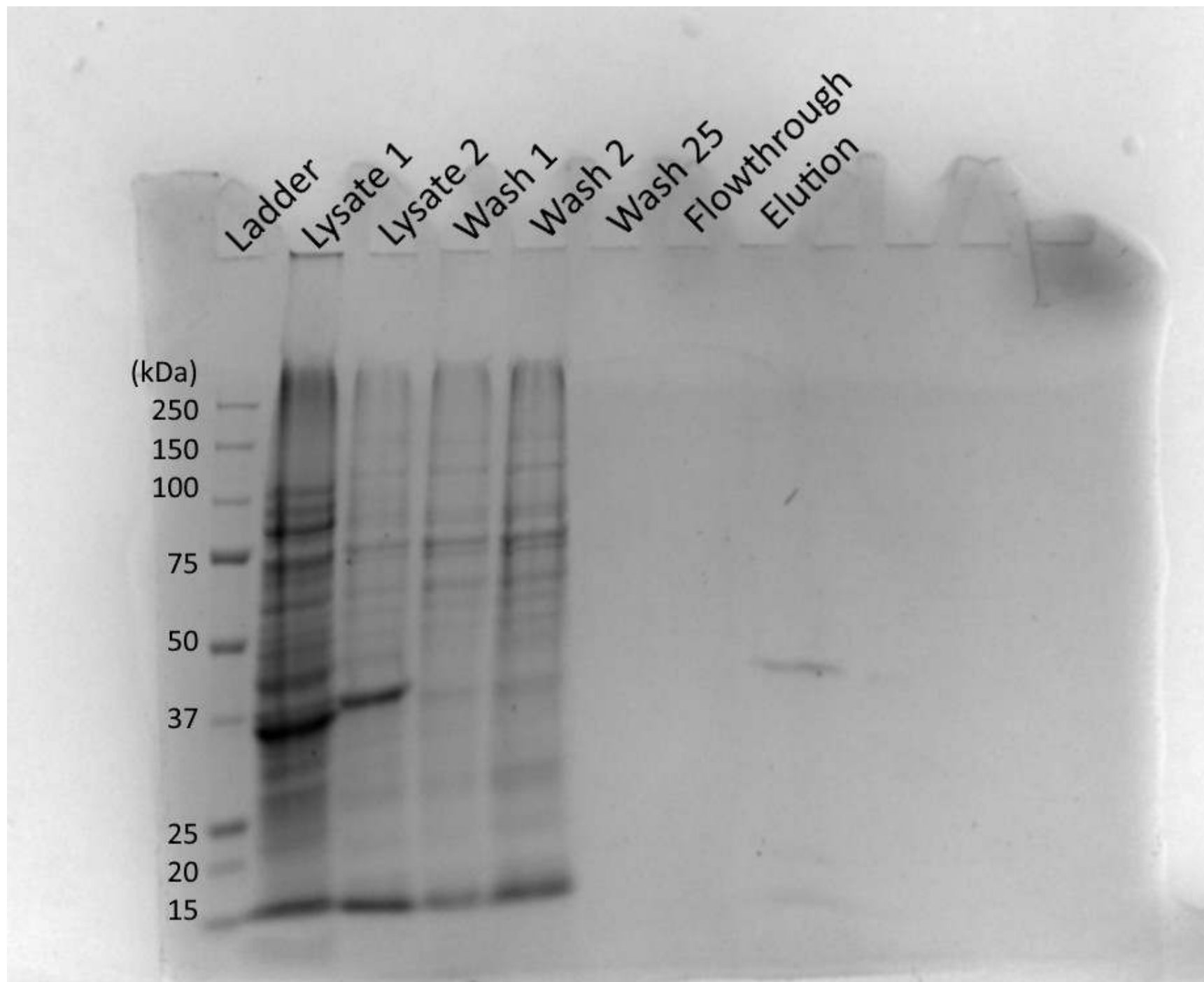


Figure 4: SDS-PAGE analysis of various fractions from purification.

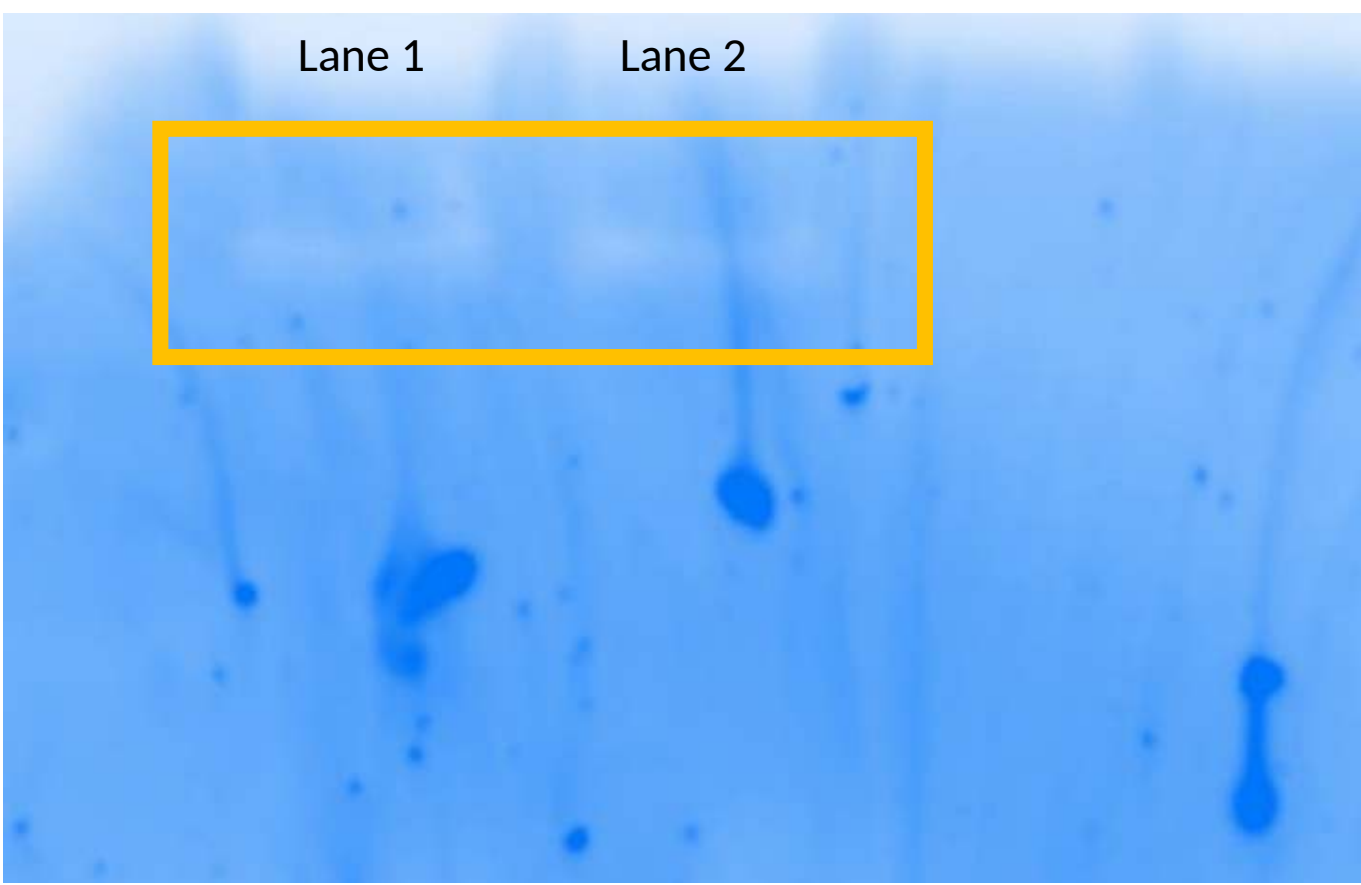


Figure 5: Gel zymography results on a 12.5% acrylamide/0.05% gelatin gel after development. [MMP-9] were ~0.148mg/ml (Lane 1) and 0.096mg/ml (Lane 2) measured by NanoDrop.

References

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3. Dennler-Church TE et al. Modification of Major Contributors Responsible for Latrine Malodor on Exposure to Hypochlorous Acid: The Potential for Simultaneously Impacting Odor and Infection Hazards to Encourage Latrine Use. *Am J Trop Med Hyg*. 2020 Dec;103(6):2584-2590.
4. Nedelea AG, Plant RL, Robins LI, Maddocks SE. Testing the efficacy of topical antimicrobial treatments using a two- and five-species chronic wound biofilm model. *J Appl Microbiol*. 2022;132(1):715-24.