Engineering of PET Hydrolase for Plastic Recycling and Environmental Remediation, and Engineering of Plastic Binding Domains for Detection and Quantification of Particles

Division of Advanced Molecular Science
(Department of Life and Coordination-Complex Molecular Science, Biomolecular Functions)

NAKAMURA, Akihiko
Professor
(Cross Appointment)
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[aki-naka@ims.ac.jp]

Education

2009	B.S. The University of Tokyo
2014	Ph.D. The University of Tokyo

Professional Employment

1995	Postdoctoral Fellow, The University of Tokyo
2015	Assistant Professor, Okazaki Institute for Integrative Bioscience
2018	Assistant Professor, Institute for Molecular Science
2020	Tenure-track Associate Professor, Shizuoka University
2022	Associate Professor (Cross Appointment), Institute for Molecular Science
2025	Professor, Shizuoka University
2025	Professor (Cross Appointment), Institute for Molecular Science

Awards

2015	HAMMON President Choice
2019	ATI Research Encouragement Award
2019	Early Career Award in Biophysics
2021	8th Biophysics and Physicobiology Editors' Choice Award
2024	Early Career Award in Glycoscience

Member
Secretary
NOMURA, Junko
KAWAGUCHI, Ritsuko

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Plastic is an indispensable material in our daily lives. It is used in a wide variety of products because it is low-cost, mass-producible, and easy to mold and process. However, due to its convenience, the amounts of plastic used and disposed are increasing every year, raising concerns about its environmental impact and sustainability. Therefore, there is a need to develop efficient and environmentally friendly recycling methods, as well as simple and rapid methods to detect plastics that have leaked into the environment.

We are attempting to obtain more active mutants by exhaustively mutating amino acid residues on the enzyme surface and screening their activity using a dispensing robot. We are also attempting to create plastic adsorption domains by modifying the amino acids constituting the adsorption surface of the carbohydrate binding domain and using a phage display method.

These studies will promote the recycling of used plastics by enzymatic degradation and promote more sustainable use of plastics. In addition, by creating a protein that detects and stains plastics, we will contribute to the protection of the natural environment by monitoring the small plastics runoff into the environment (Figure 1).

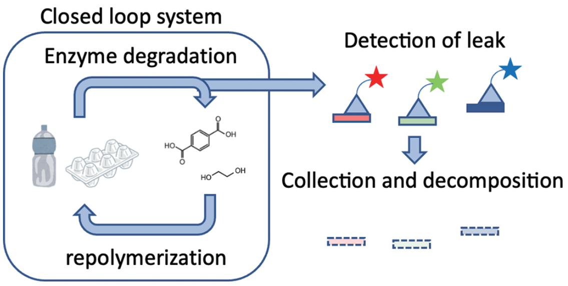

Figure 1. Scheme of plastic recycling and detection system.

Selected Publications

- T. Matsuzaki, T. Saeki, F. Yamazaki, N. Koyama, T. Okubo, D. Hombe, Y. Ogura, Y. Hashino, R. Tatsumi-Koga, N. Koga, R. Iino and A. Nakamura, "Development and Production of Moderate-Thermophilic PET Hydrolase for PET Bottle and Fiber Recycling," *ACS Sustainable Chem. Eng.* **13**, 10404–10417 (2025).
- Y. Ogura, Y. Hashino and A. Nakamura, "Direct Screening of PET Hydrolase Activity in Culture Medium Based on Turbidity Reduction," *ACS Omega* **9**, 34151–34160 (2024).
- A. Nakamura, N. Kobayashi, N. Koga and R. Iino, "Positive Charge Introduction on the Surface of Thermostabilized PET Hydrolase Facilitates PET Binding and Degradation," *ACS Catal.* **11**, 8550–8564 (2021).
- A. Nakamura, D. Ishiwata, A. Visootsat, T. Uchiyama, K. Mizutani, S. Kaneko, T. Murata, K. Igarashi and R. Iino, "Domain Architecture Divergence Leads to Functional Divergence in Binding and Catalytic Domains of Bacterial and Fungal Cellobiohydrolases," *J. Biol. Chem.* **295**, 14606–14617 (2020).
- A. Nakamura, K. Okazaki, T. Furuta, M. Sakurai and R. Iino, "Processive Chitinase Is Brownian Monorail Operated by Fast Catalysis after Peeling Rail from Crystalline Chitin," *Nat. Commun.* **9**, 3814 (2018).

1. Development and Production of Moderate-Thermophilic PET Hydrolase for PET Bottle and Fiber Recycling

We developed a highly efficient PET hydrolase variant, PET2-21M, that functions under moderate temperature conditions and enables practical enzymatic recycling of PET bottles and blended fibers.[1)] PET recycling is an urgent challenge, since mechanical methods usually yield downgraded materials and chemical recycling requires harsh reagents and conditions. Enzymatic approaches offer a sustainable alternative, but natural PET hydrolases typically show low activity at moderate temperatures, limiting their industrial applicability. We aimed to engineer a robust enzyme with high catalytic efficiency, thermostability, and broad substrate scope.

We started from our previously optimized variant, PET2-7M, and introduced additional beneficial mutations using random mutagenesis, rational design, and structural insights. We identified seven novel mutations that enhanced activity, and we further modified surface charges to improve PET binding. Inspired by structural features of HotPETase, we redesigned the substrate-binding cleft. By combining these improvements, we created PET2-14M-6Hot, and then advanced it to PET2-21M with 21 total mutations. These changes substantially increased enzyme activity and maintained stability around 60 °C, in contrast to many existing hydrolases that require higher temperatures.

We demonstrated significant performance gains with PET2-21M. Compared to the original PET2, our variant produced about 30 times more soluble degradation products. At 60 °C, in the presence of 20 g/L PET powder, PET2-21M depolymerized 95% of the substrate within 24 hours, surpassing LCC-ICCG, which required 72 °C to reach a comparable result. Even with reduced enzyme loading, PET2-21M remained highly efficient: at 2.5 mg/L, it maintained nearly half of its maximum activity, about twice that of LCC-ICCG under the same conditions. At 40 g/L PET, we achieved almost 80% conversion with 10 mg/L enzyme, and 44% with 5 mg/L, both outperforming the benchmark.

We also validated our variants on PET fibers and blended textiles. PET2-14M-6Hot produced 75.7 mM degradation products from pure PET fibers at 60 °C, exceeding the 70 °C performance of LCC-ICCG. For PET/cotton blends, we measured 62.8 mM compared to 46.7 mM for the comparator. For PET/PU blends at 50 °C, our enzyme generated 19.2 mM, more than double the 8.2 mM obtained with LCC-ICCG. These findings show that our engineered enzymes are effective not only for bottles but also for complex textile waste streams.

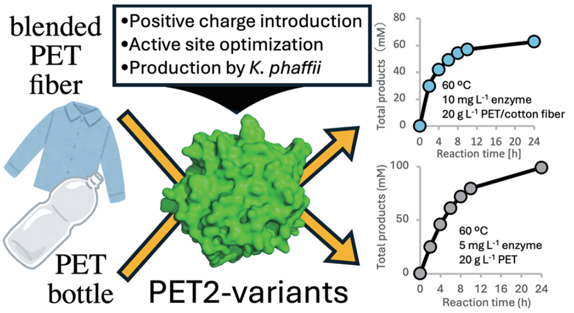

Figure 2. Scheme of PET fiber degrading enzyme development.

To ensure industrial scalability, we expressed our variants in the yeast *Komagataella phaffii*. We obtained high yields, with PET2-14M-6Hot reaching nearly 700 mg/L secretion. We also observed limited glycosylation, simplifying purification and downstream processing. These results confirm that our engineered enzymes can be produced in large amounts in microbial hosts suitable for industrial application.

In summary, we achieved a major advance in enzymatic PET recycling by creating PET hydrolases that combine high activity at moderate conditions with effectiveness on bottles and blended fibers. Our enzymes reduce energy demands, increase efficiency, and can be manufactured at scale. We believe this work provides a strong foundation for future development of cost-effective, environmentally friendly plastic recycling systems that align with circular economy goals.

2. Artificial Evolution of Carbohydrate Binding Domain to PET Binding Domain

To develop a method for detection of plastics using plastic binding proteins, we developed PET binding protein using saturation mutagenesis and M13 phage display. We have already developed 2 generations of mutants which reduced the binding affinity to chitin and cellulose. Thus, we also checked the binding properties of the mutants against PET powder.

The binding affinities of six 2nd generation mutants (LWL to WVF) and one 1st generation mutant (4M) were compared with that of the template protein from *Pyrococcus furiosus* (WT). LWL showed 2.5 times higher binding amount (0.10 nmol) and similar dissociation constant (3.1 mg/mL) than WT (0.041 nmol and 3.2 mg/mL). We started to determine the binding and dissociation rate constants of LWL mutant and WT by single molecule observation for revealing the improved parameter of LWL mutant.

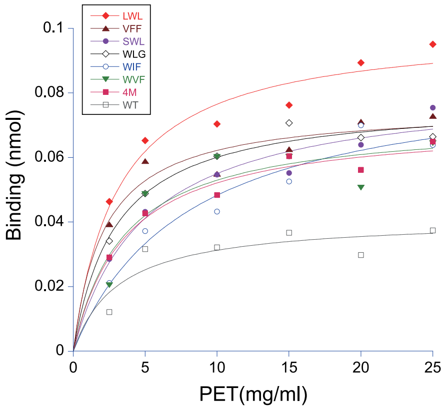

Figure 3. Binding measurement of 2nd generation of mutants against PET powder.

Reference

1) T. Matsuzaki, T. Saeki, F. Yamazaki, N. Koyama, T. Okubo, D. Hombe, Y. Ogura, Y. Hashino, R. Tatsumi-Koga, N. Koga, R. Iino and A. Nakamura, *ACS Sustainable Chem. Eng.* **13**, 10404–10417 (2025).