Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information

Heterogeneous Nanozymatic Activity of Hf Oxo-Clusters Embedded in a Metal-Organic Framework Toward Peptide Bond Hydrolysis

Jens Moons[1], Alexandra Loosen[1], Charlotte Simms[1], Francisco de Azambuja[1], Tatjana N. Parac-Vogt[1*]

[1] *Department of Chemistry, KU Leuven, Celestijnenlaan 200F,3001 Leuven, Belgium*

*tatjana.vogt@kuleuven.be

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Materials and methods

^{1}H NMR spectra were recorded with a Bruker Avance 400 spectrometer in deuterated solvents and with 0.1 M TMSPA-d_4 as an internal reference. Spectra were analyzed using Topspin software. Powder X-ray diffraction (PXRD) patterns were collected on a Malvern PANalytical Empyrean diffractometer (in transmission mode) over a 1.3 – 45° 2θ range, using a PIXcel3D solid state detector and Cu anode (Cu $K_{\alpha1}$: 1.5406 Å; Cu $K_{\alpha2}$: 1.5444 Å). Scanning electron microscopy (SEM) micrographs were recorded using a JEOL-6010LV SEM after depositing a palladium/gold layer on the samples with a JEOL JFC-1300 autofine coater under Ar plasma. N_2 physisorption isotherms were measured on a Micromeritics 3Flex surface analyzer at -196 °C. Prior to measurements, samples were evacuated at 120 °C under vacuum for 12 h. Surface areas were calculated using the multi-point BET method applied to the isotherm adsorption branch taking into account the Rouquerol consistency criteria and the micropore volume was calculated at $P/P_0 = 0.5$.[1] Inductively coupled plasma optical emission spectrometry (ICP-OES) was measured on a PerkinElmer optical emission spectrometry Optima 8300 instrument.

1,3,6,8-tetrabromopyrene (97 %, TCI), 4-ethoxycarbonylphenylboronic acid (TCI), K_3PO_4 (Sigma Aldrich), $Pd(PPh_3)_4$ (Sigma Aldrich), 1,4-dioxane (99.5 %, Acros Organics), acetone (Fisher Scientific), chloroform (Fisher Scientific), methanol (Fisher Scientific), KOH (Acros Organics), HCl (37 %, ChemLab), $HfOCl_2.8H_2O$ (98 %, Alfa Aesar), benzoic acid (Sigma Aldrich), *N,N*-dimethylformamide (Fischer Scientific), D_2SO_4 (Sigma Aldrich), NaOD (40 wt%, Sigma Aldrich), TMSPA-d_4 (Sigma Aldich), DMSO-d_6 (Sigma Aldrich). All chemicals were obtained from commercial sources and used without further purification.

Synthesis procedures

H_4TBAPy linker was synthesized according to published methods via standard Suzuki-Miyaura reaction between 1,3,6,8-tetrabromopyrene and 4-ethoxycarbonylphenylboronic acid with some modifications.[2] The ester is saponified by excess of base to get full conversion of the ester and after acidification the carboxylate linker is obtained with an overall yield of 47 %. The Hf-MOF NU-1000 was synthesized according to literature.[3] NU-1000 is prepared via an upscaled solvothermal method in which hafnium oxychloride octahydrate is mixed with the H_4TBAPy linker in *N,N*-dimethylformamide with benzoic acid as modulator.[3] The modulator is removed after synthesis by washing with a 37 % HCl solution. After thermal activation (20h at 120 °C), the desired product is characterized with powder X-ray diffraction, scanning electron microscopy, thermogravimetric analysis and N_2 physisorption.

Synthesis of tetraethyl-4,4′,4″,4‴-(pyrene-1,3,6,8-tetrayl)tetrabenzoate (1)

Under N_2 atmosphere, 4 g of 1,3,6,8-tetrabromopyrene (7.72 mmol), 6.6 g of 4-ethoxycarbonylphenylboronic acid (34.02 mmol), 13.2 g of K_3PO_4 (62.18 mmol) and 0.6 g of $Pd(PPh_3)_4$ (0.52 mmol) were added to 216 mL 1,4-dioxane under continuous stirring. The suspension is stirred for 48-72 h at 90 °C under N_2 atmosphere. Over time the solution becomes more and more yellow and turns black when the reaction is complete. 160 mL of H_2O is added

and the mixture is cooled down. The solution is filtered and the yellow precipitate is washed twice with 80 mL of H_2O and twice with 160 mL of acetone. The precipitate is then dissolved in 240 mL of boiling chloroform. Under vacuum, the volume is reduced by half and subsequently 240 mL of methanol is added. After 30 minutes a yellow precipitate is recovered by centrifugation and dried at 70 °C under vacuum for 12 h (yield: 3.17 g; 52 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.25-8.23 (d, 8H, phenyl H), 8.15 (s, 4H, pyrene H), 8.01 (s, 2H, pyrene H), 7.76-7.74 (d, 8H, phenyl H), 4.48-4.43 (q, 8H, CH_3-C**H**$_2$-O), 1.55-1.44 (t, 12H, C**H**$_3$-CH_2-O).

Synthesis H_4TBAPy linker: saponification of (1)

3.17 g of (1) (3.99 mmol) was added to 160 mL 1,4-dioxane. To this suspension 220 mL of an aqueous KOH solution (0.4 M) was added and the mixture was then refluxed for 24 h. The precipitate is filtered while hot to remove the greyish residue. After cooling down and upon adding 30 mL of HCl 37 % a yellow precipitate is formed. This precipitate is centrifuged and washed 3 times with 100 mL of H_2O. The product is dried overnight at 100 °C under vacuum and then for 3 h at 80 °C, also under vacuum. (yield: 2.45 g; 90 %)

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 13.11 (br, 4H, COOH), 8.20-8.15 (s+d, 12H, pyrene+phenyl H) 8.08 (s, 2H, pyrene), 7.87-7.85 (d, 8H, phenyl H).

Synthesis of Hf-NU-1000[3]

2.0 g of $HfOCl_2.8H_2O$ (4.9 mmol) and 43.2 g of benzoic acid (35.4 mmol) are added together in 128 mL of *N*,*N*-dimethylformamide (DMF) in a 500 mL VWR pressure plus+ Schott bottle, sonicated until fully dissolved and put in an oven at 80 °C for 1 h. Next, the bottle is removed from the oven and 480 mg of H_4TBAPy (0.703 mmol) is added. The mixture is sonicated till a fine solid suspension is observed, and then put in an oven at 100 °C. After 24 h, a clear solution is obtained. After cooling down, the precipitated product is isolated by centrifugation of the reaction mixture. The solid product was washed 2 times with 25 mL of DMF, each time soaking for 2 h. After centrifugation, the precipitate is added to 100 mL of DMF in a 500 mL VWR pressure plus+ Schott bottle and 8 mL of HCl (8 M) is added dropwise while gently shaking. This mixture is placed in a 100 °C oven for 18 h. After cooling down, the precipitated product is isolated by centrifugation of the reaction mixture, and washed 3 times with 25 mL of DMF for 2 h each portion, and 3 times with 40 mL of acetone, each time soaking for 2 h , followed by a final wash with 40 mL of acetone for 18 h. The product is dried at 80 °C under vacuum for 1 h (yield: 0.85 g; 90 %). Before hydrolysis experiments, Hf-NU-1000 was activated at 120 °C for 18 h.

Hydrolysis studies of dipeptides.

900 µL of D_2O was mixed with 5.3 mg Hf-NU-1000 (2 µmol of Hf_6 clusters) and 100 µL of a 20 mM dipeptide solution was added. pD values were adjusted to the required value with NaOD. Reactions were performed in individual vials at 60 °C and at certain time points, the solution of a vial was centrifuged at 14000 rpm for 2 x 10 min, after which the supernatant solution was sampled for analysis. Temperature dependence studies followed the

same procedure with triplicate reactions performed at 37, 50, 60 and 80 °C. For pD dependence, reactions were carried out in a pD range between 3.4 and 9.4 with intervals of one, each after 24 hours of reaction at 60 °C. All the reactions (500 µl) were followed with ^{1}H NMR spectroscopy and 5 µl of a 0.1 M 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSPA-d_4) solution was added to the supernatant before measurements. Reaction rates were determined by fitting a first-order decay function to the data. All k_{obs} are calculated averages of three replicates.

Hydrolysis of proteins and SDS-PAGE.

950 µL of H_2O was added to 2 µmol Hf-NU-1000 and mixed with 50 µL of 0.4 mM hen egg white lysozyme (HEWL) solution. The pH of the mixture was adjusted to 7.0 and reactions were run at 60 °C. All different analysed time points were run in a separate reaction vial. Samples of 15 µL of the reaction mixture were mixed with 5 µL sample buffer. Samples were run on 18 % Laemmli gels in a OmniPAGE electrophoretic cell at 200 V for 1.5 h. Page Ruler unstained low range protein ladder was used as a standard. Silver staining was used for imaging the gel and analysis was done with ImageLab.

Recycling experiment.

Reactions were prepared as described above. After 24h at 60°C, the catalyst was recovered by centrifugation and regenerated by overnight stirring in D_2O. Hf-NU-1000 was then recovered by centrifugation for the next cycle. five reaction cycles of 24h with 2 mM GG were performed following the above procedure. Conversion rates were calculated from ^{1}H-NMR analysis of the supernatant whereas stability of the Hf-NU-1000 MOF was evaluated by powder X-ray diffraction.

Supplementary figures

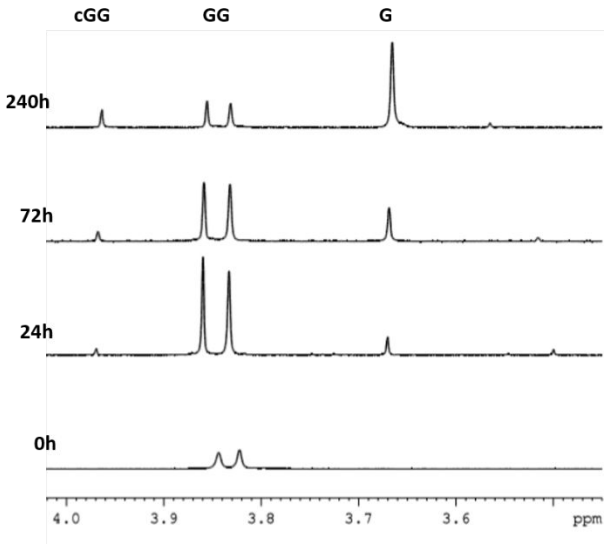

Figure S1 NMR profile of 2 mM GG hydrolysis reaction over time in presence of 2 μmol Hf-NU-1000 at 60 °C and pH 7.4.

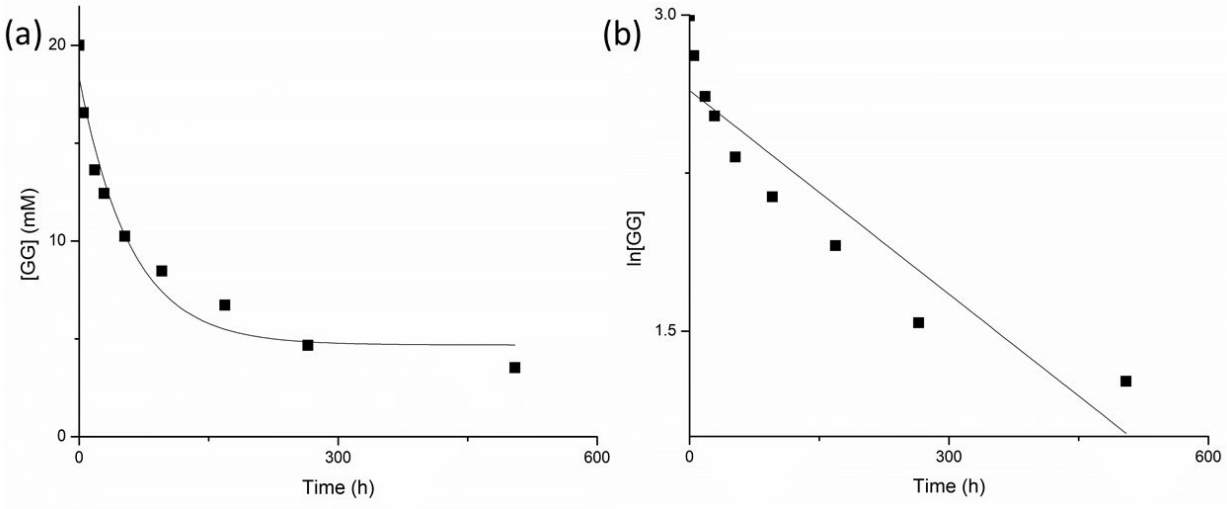

Figure S2 Reaction of 20 mM GG with 2 μmol Hf-NU-1000 at 60 °C and pD 7.4. (a) Concentration of GG as a function of time by ^{1}H NMR. (b) First order decay fit of ln[GG] as a function of time for the reaction of 20 mM GG with 2 μmol Hf-NU-1000 at 60 °C and pD 7.4.

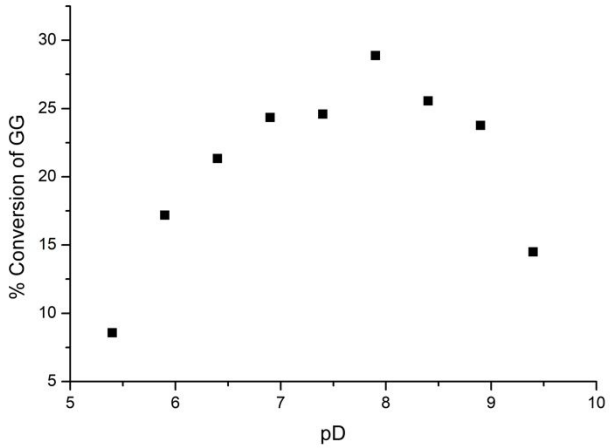

Figure S3 Influence of pD on the conversion rate of 2 mM GG to G in presence of 2 µmol Hf-NU-1000, 60 °C, 24 hours.

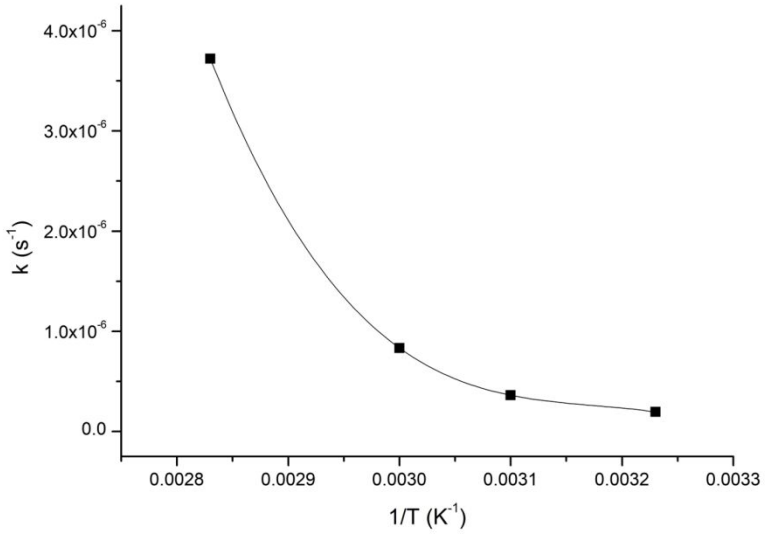

Figure S4 Reciprocal temperature dependence on the reaction rate of 2 mM GG in presence of 2 µmol Hf-NU-1000 at pH 7.4.

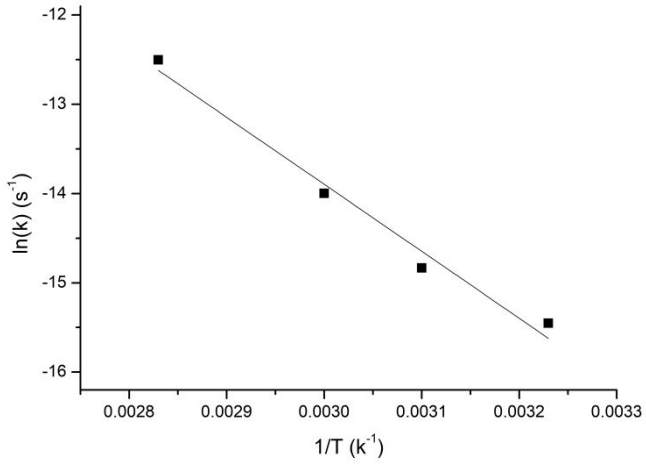

Figure S5 Arrhenius plot of the reaction rate of 2 mM GG and 2 μmol Hf-NU-1000 at pH 7.4, as a function of reciprocal temperature. Linear fit: Y = -7505.55*X + 8.62 (R^2 = 0.97297).

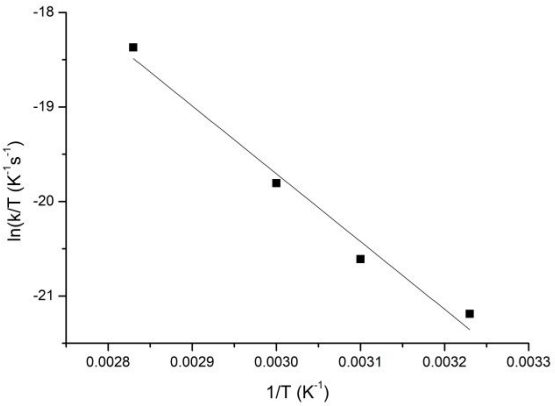

Figure S6 Plot depicting ln(k/T) as a function of reciprocal temperature for 2 mM GG in the presence of 2 μmol Hf-NU-1000 at pH 7.4. Linear fit: Y = -7174.72*X + 1.81 (R^2 = 0.97084).

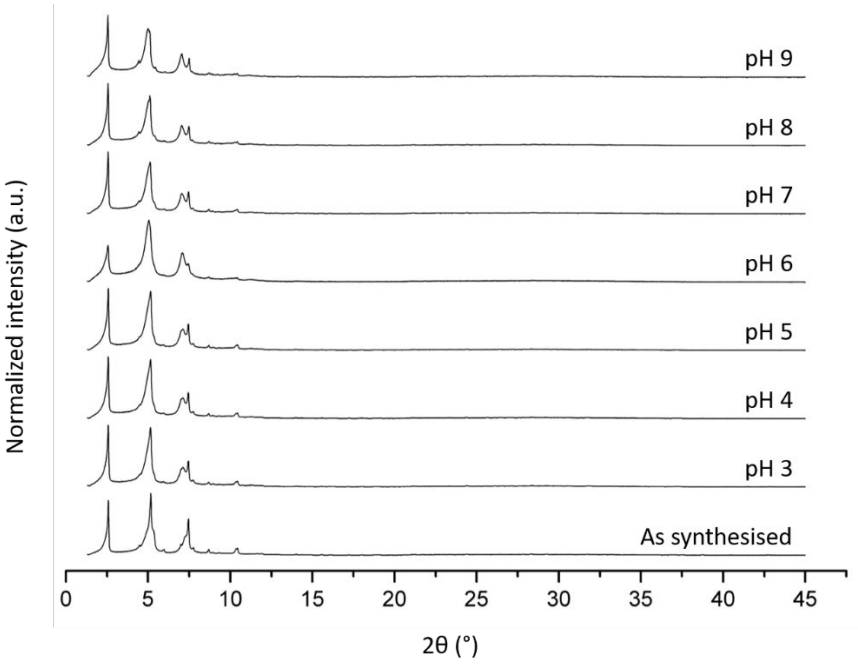

Figure S7 PXRD patterns of Hf-NU-1000 as synthesised and after incubation with GG at 60 °C for 24 hours at different pH values.

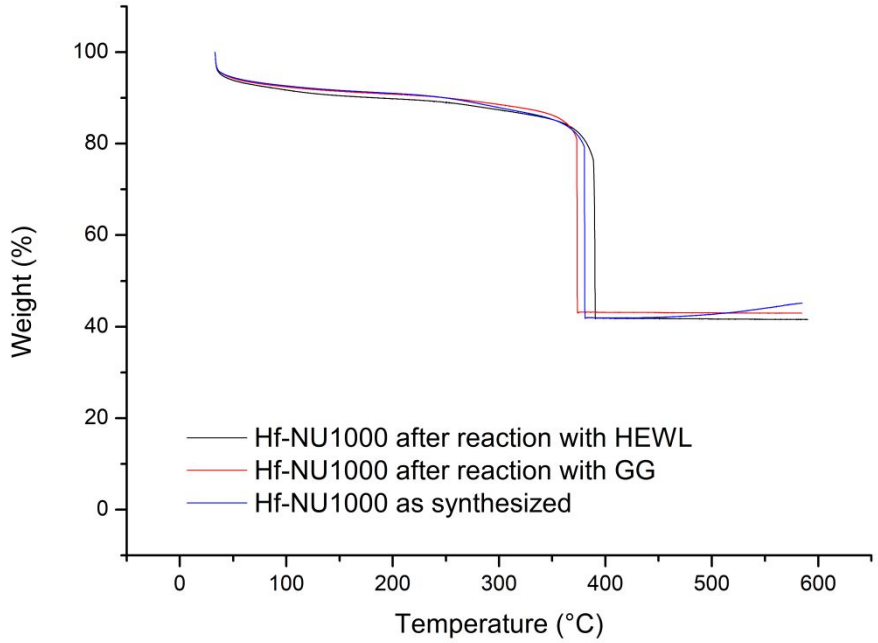

Figure S8 TGA analysis of Hf-NU-1000 as synthesized or after reaction with HEWL, GG.

References

(1) Rouquerol, J.; Rouquerol, F.; Sing, K. S. W. *Absorption by Powders and Porous Solids; 2nd Edition*; Academic Press, 2013.

(2) Wang, T. C.; Vermeulen, N. A.; Kim, I. S.; Martinson, A. B. F.; Fraser Stoddart, J.; Hupp, J. T.; Farha, O. K. Scalable Synthesis and Post-Modification of a Mesoporous Metal-Organic Framework Called NU-1000. *Nat. Protoc.* **2016**, *11* (1), 149–162. https://doi.org/10.1038/nprot.2016.001.

(3) Beyzavi, M. H.; Klet, R. C.; Tussupbayev, S.; Borycz, J.; Vermeulen, N. A.; Cramer, C. J.; Stoddart, J. F.; Hupp, J. T.; Farha, O. K. A Hafnium-Based Metal–Organic Framework as an Efficient and Multifunctional Catalyst for Facile CO2 Fixation and Regioselective and Enantioretentive Epoxide Activation. *J. Am. Chem. Soc.* **2014**, *136* (45), 15861–15864. https://doi.org/10.1021/ja508626n.