

Metal-oxo clusters and metal organic frameworks as nanozymes

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LABORATORY OF BIOINORGANIC CHEMISTRY

KU Leuven, Belgium

We are located at the heart of Belgium, in one of the oldest European universities in the beautiful city of Leuven.

Our interdisciplinary research is at the interface of inorganic chemistry, biochemistry, materials science and catalysis.

We exploit metal cluster based complexes and materials such as polyoxometalates (POMs) and metal-organic frameworks (MOFs) for biologically inspired reactivity with biomolecules and model systems. We also create new hybrid structures based on polyoxometalates using principles of supramolecular chemistry and biomolecular recognition.

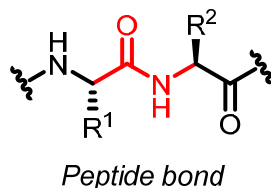
OUR RESEARCH



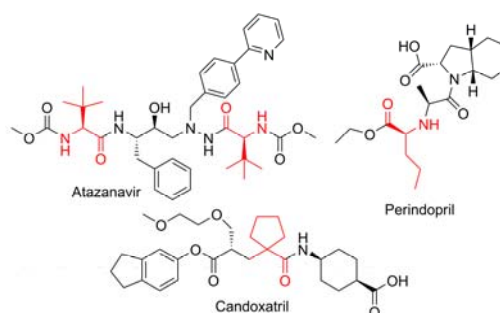
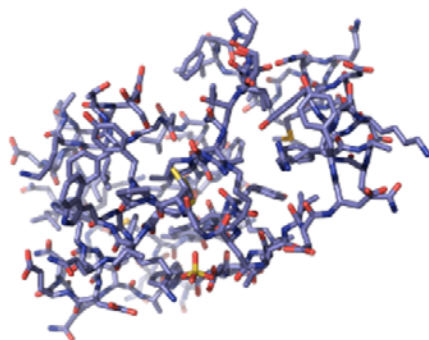
@TPV_group

Peptide Bond

An important sub-group of amide bonds widely present in biomolecules and bioactive compounds



Peptide bonds form the backbone of proteins and are essential part of therapeutic peptides



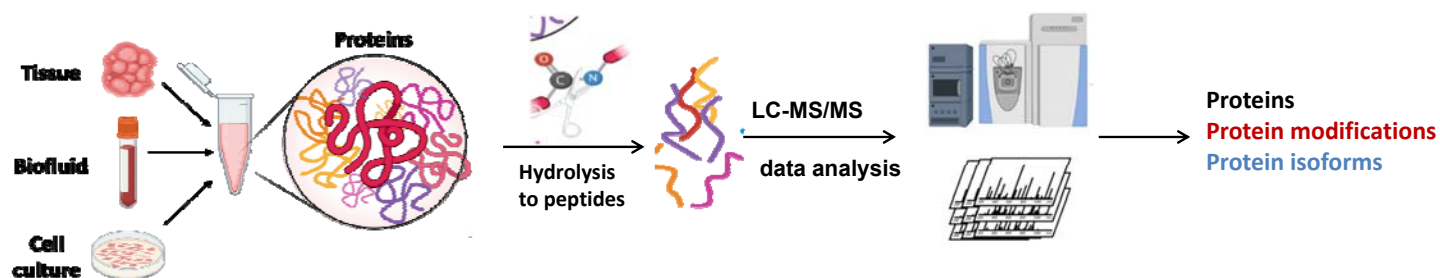
5

Why breaking peptide bonds?

The ability to rationally manipulate and control the fragmentation of large proteins remains a challenge in the field of biochemistry, biotechnology and proteomics.

Selective cleavage of proteins is one of the most required and most important procedures in proteomics, which is the large-scale study of proteins' structure and function.

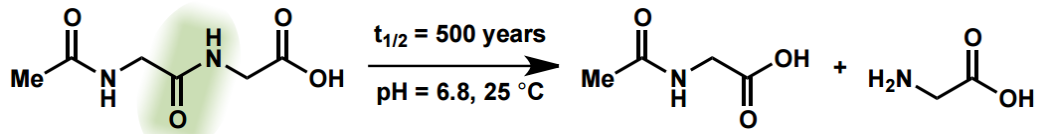
Protein modifications and protein isoforms are linked to many diseases such as cancer, auto-immune and neurological disorders.



Proteomics market budget:

7.9 billion USD (2009)
20.6 billion USD (2018)
46.7 billion USD (2025)

Hydrolysis of peptide bond



Nearly 3000 years needed for the full hydrolysis of the peptide bond

Proteolytic enzymes: Trypsin

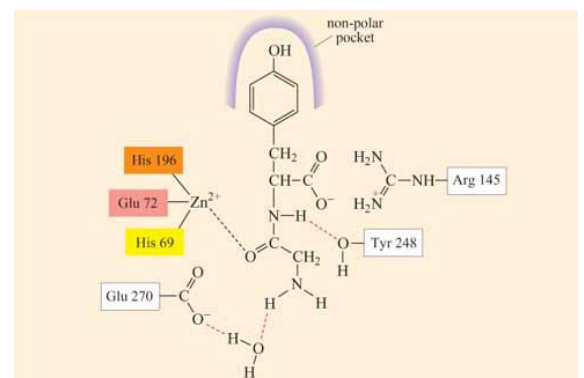
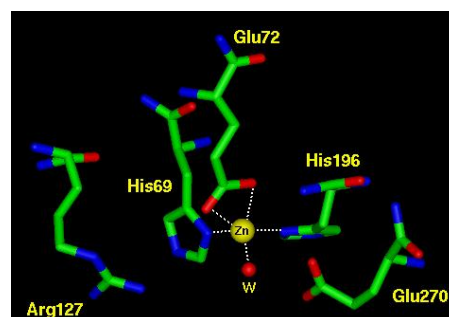
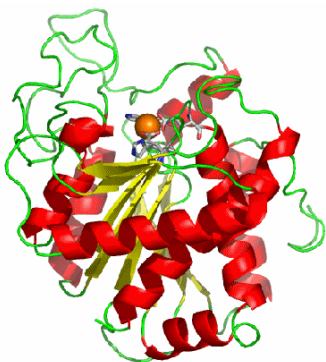
- Efficient
- Selective
- Expensive
- Sensitive to reaction conditions
- Self-Digestion
- Difficult to tune the reactivity and selectivity
- >50 % of created protein fragments are 6 amino acids or shorter

Need for chemical catalyst that mimic natural proteases

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Metalloproteases

Carboxypeptidase A (CPA)



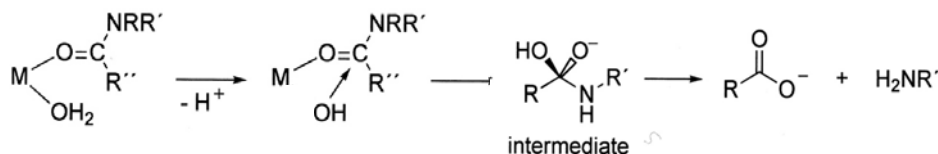
Metal Complexes?

Requirements: **Reactivity AND Selectivity**

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Metal Complexes as artificial proteases

Reactivity



Interaction with the metal ion polarizes C=O group in the peptide bond making it more susceptible for nucleophilic attack by H₂O/OH

Which metals are the best candidates?

- High Lewis acidity
- Redox inactive
- High coordination numbers and flexible geometries
- Kinetically labile and rapid ligand-exchange kinetics
- Oxophilic

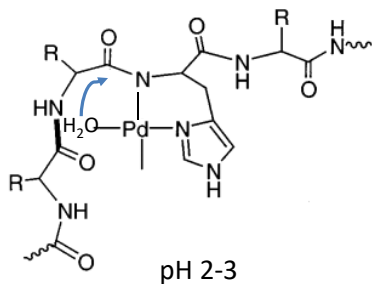


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Metal complexes as artificial metalloproteases

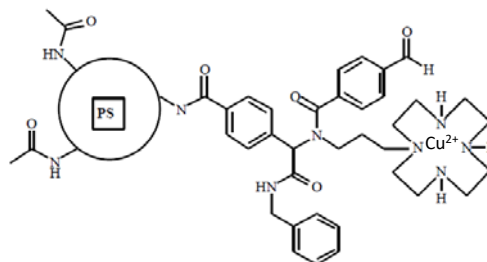
How to induce Selectivity?

Coordination of Pd(II) to the side chains of His and Met in proteins



Parac et al., *J. Am. Chem. Soc.*, 1996, 118, 51.
Parac et al., *J. Am. Chem. Soc.*, 1996, 118, 5946.
Parac et al., *J. Am. Chem. Soc.*, 1999, 121, 3127.

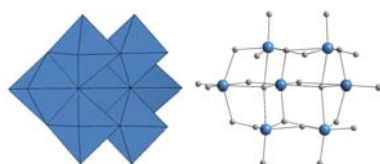
Hydrophobic interactions between polystyrene-conjugated Cu(II) complex and proteins



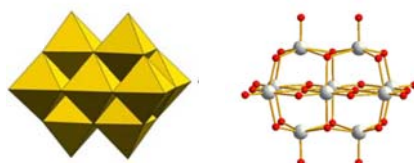
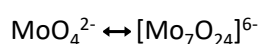
Yoo et. al. *J. Am. Chem. Soc.*, 2003, 125, 14580.
Yoo et. al. *J. Am. Chem. Soc.*, 2005, 127, 9593.

Polyoxometalates (POMs) as an alternative?

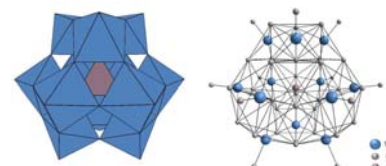
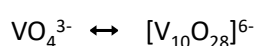
A large class of **discrete, water soluble, negatively charged**, nano-sized metal-oxygen clusters, formed by early transition metals in their highest oxidation state



Heptamolybdate $[\text{Mo}_7\text{O}_{24}]^{6-}$



Decavanadate $[\text{V}_{10}\text{O}_{28}]^{6-}$



Keggin POM: $[\text{PW}_{12}\text{O}_{40}]^{3-}$

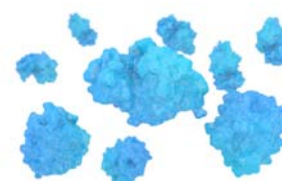
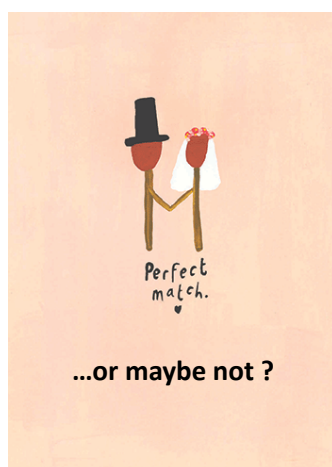
Versatility in **shape, size, charge, polarity** allows for tuning POMs interaction with the **positively charged surfaces**

Polyoxometalates and Proteins: do opposites attract?



Polyoxometalates

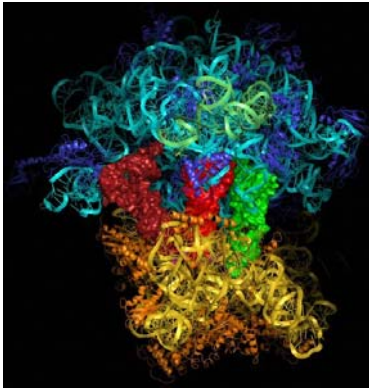
- “Hard” inorganic clusters
- Highly symmetrical
- Good solubility in various solvents
- Negative surface charges



Proteins

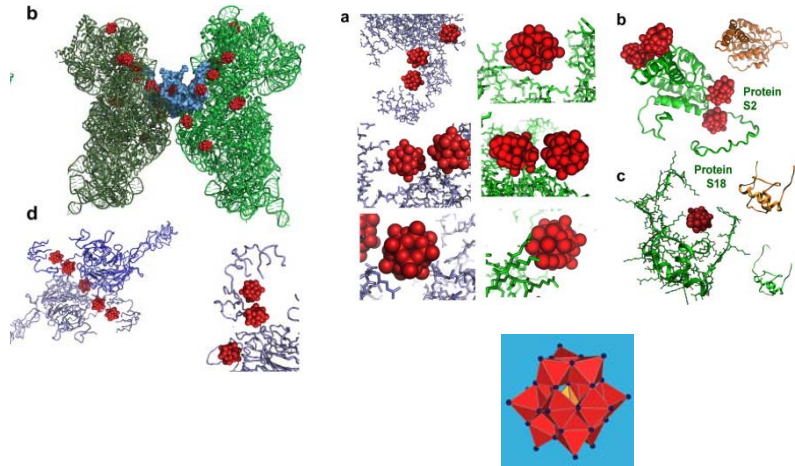
- “Soft” biomolecules
- Heterogeneous shapes and sizes
- Prefer aqueous environment
- Positive/negative/neutral surface charges

- POMs have a broad range of anti-viral, anti-bacterial, anti-tumor, anti-fungal activities
- Biological activity is often linked to specific interactions of POMs with protein surface
- POMs are frequently used as additives in protein crystallography



X-ray structure of Ribosome

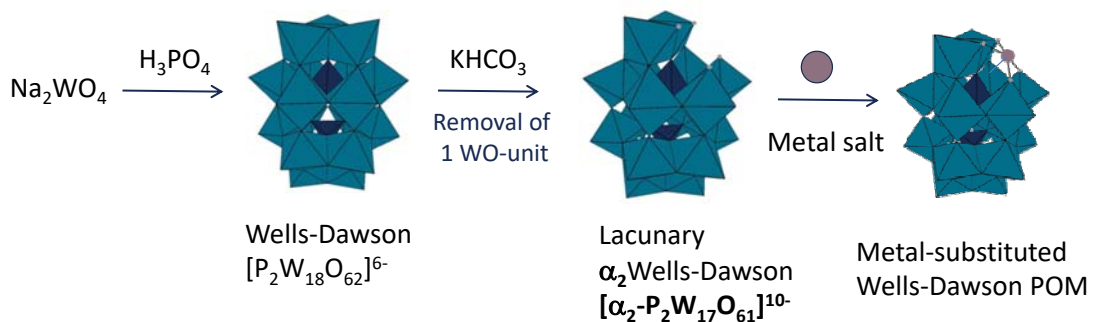
Ada E. Yonath
Nobel Prize Chemistry 2009



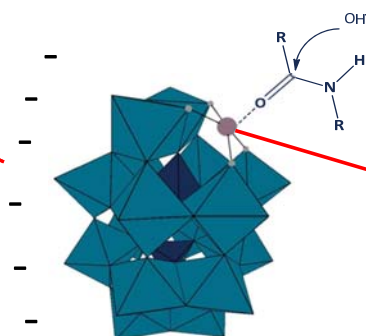
Keggin POM: $[PW_{12}O_{40}]^{3-}$

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Design of POMs as artificial proteases

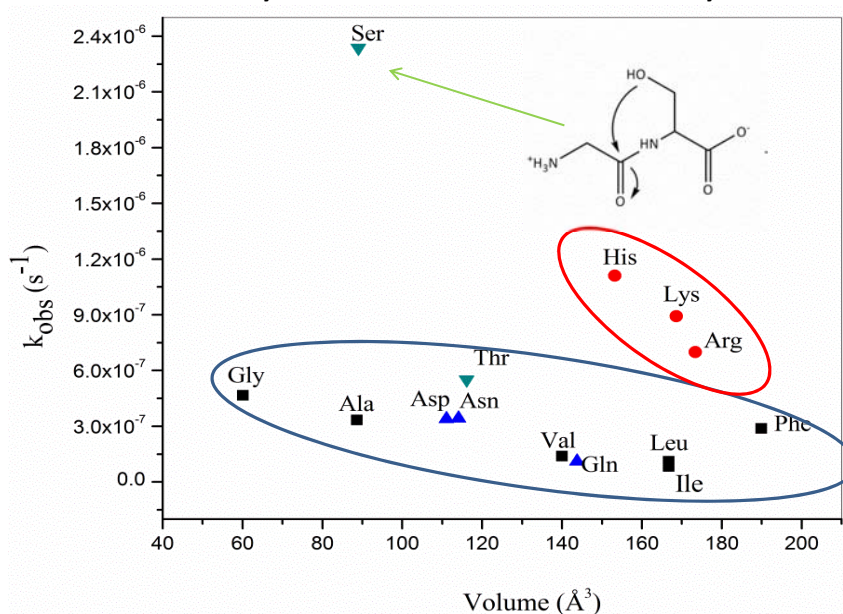
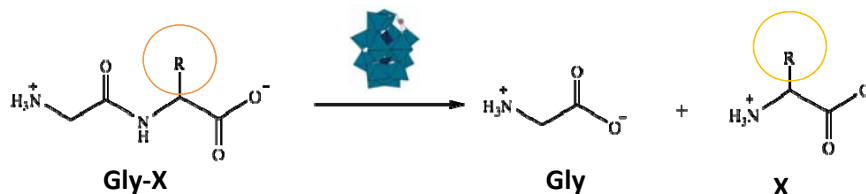


Specific
Interaction with
positive patches on
protein surface
(Lock & Key model)



Lewis acid
metal Ion (Zr,Hf) as
catalytic site

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Interaction between the positively charged side chain and negatively charged POM results in a more stable complex

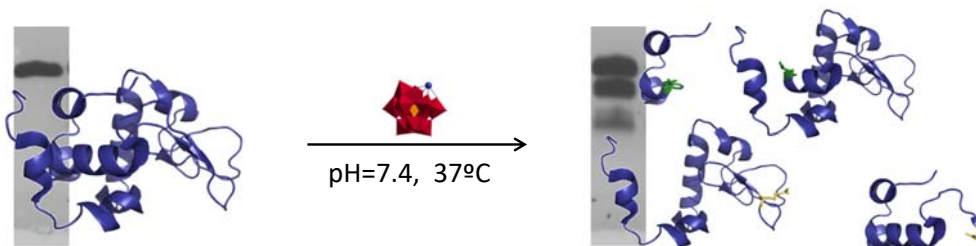
Influence of steric effects

H.G. T. Ly, T.Mihaylov, G. Absillis, K. Pierloot, T. N. Parac-Vogt, *Inorg. Chem.*, **2015**, 54,11477.

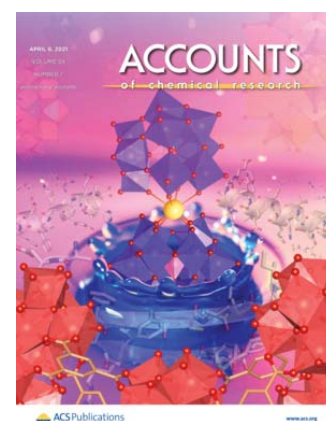
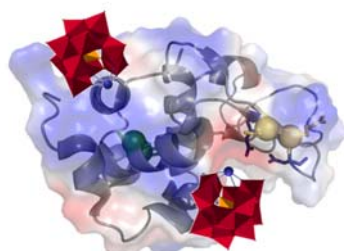
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Can Zr-POMs hydrolyze proteins?

1) HYDROLYSIS (SDS-PAGE, Image Lab kinetics, Edman degradation, ESI-MS)



2) INTERACTION (CD, ITC, ^{31}P NMR, ^1H , ^{15}N -HSQC NMR, luminescence, fluorescence, X-ray spectroscopy and theoretical modeling)

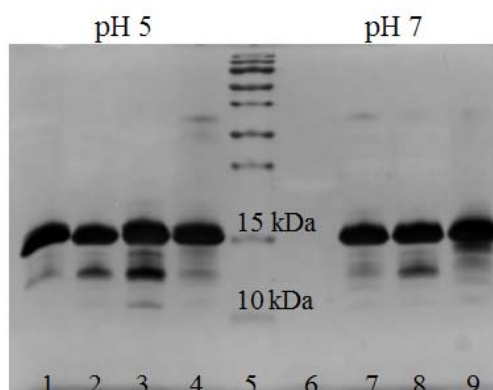
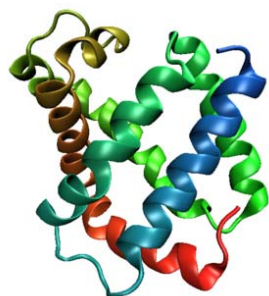


F. de Azambuja, J. Moons,
T. N. Parac-Vogt
Acc. Chem. Res., **2021**, 54, 1673

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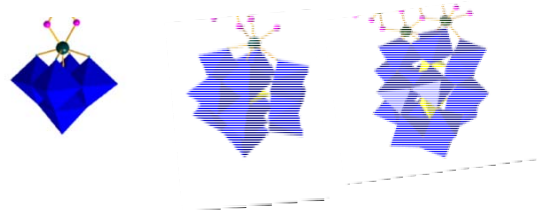
Hydrolysis of Myoglobin by Zr-POMs

Helical structure, 153 Amino acids



Structure-Activity Relationship:

Different POMs = Same Selectivity
Reactivity → Size, Shape, Charge of POM



- The same fragmentation pattern was observed in the presence of all Zr-POMs
- Different yields indicate the importance of POM/protein lock-key interaction for the efficiency of hydrolysis

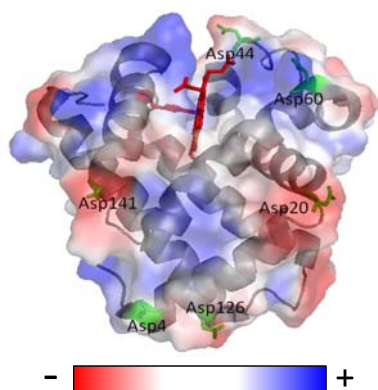
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Selectivity?

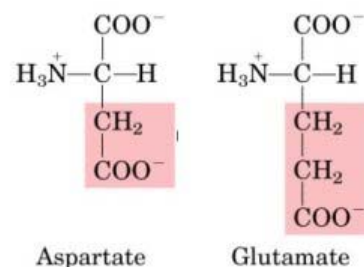
“Give me reactivity, and I will give you selectivity later”

B. Sharpless, Nobel laureate 2001,2022

Edman degradation, LC-MS/MS → Cleavage Sites



Asp4-Gly5
Asp20-Ile21
Asp44-Lys45
Asp60-Leu61
Asp126-Ala127
Asp141-Ile142



Aspartate

Glutamate

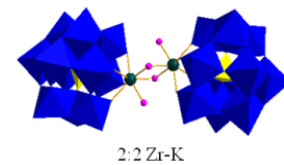
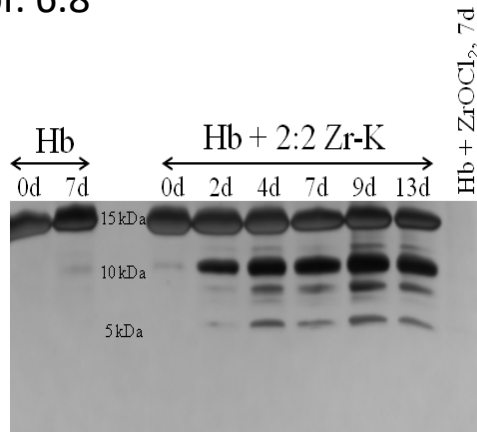
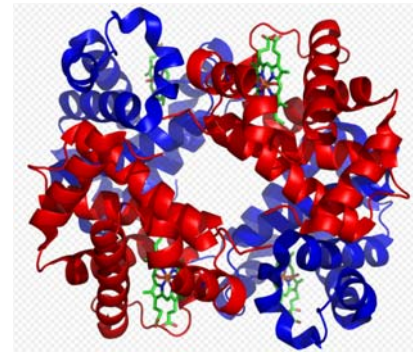
All hydrolyzed peptide bonds are located in the near vicinity of a positively charged surface patch that can electrostatically interact with the negatively charged POM surface

H. G. T. Ly, G. Absillis, R. Janssens, P. Proost, T. N. Parac-Vogt
Angew. Chem. Int. Ed. **2015**, 25, 7391.

Hydrolysis of Bovine Hemoglobin

Bovine Hb (64.5 kDa): 572 amino acids

- 2 α subunits: 141 residues, 15.04 kDa
- 2 β subunits: 145 residues, 15.94 kDa
- pI: 6.8



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Exclusively Asp-X sequence hydrolyzed!

α -chain

Asp6-Lys7
 Asp74-Asp75
 Asp75-Leu76
 Asp85-Leu86
 Asp94-Pro95
 Asp116-Phe117

β -chain

Asp46-Leu47
 Asp51-Ala52
 Asp68-Ser69
 Asp78-Asp79
 Asp98-Pro99

α -chain

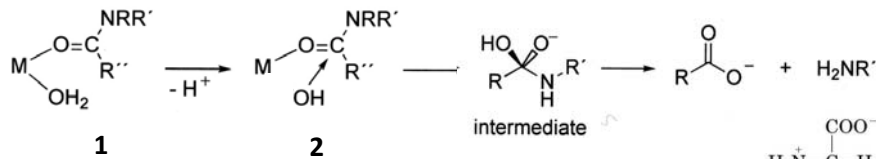
VLSAADKGNV KAAWGVKGGH AA EYGAEALE RMFLSPTTK TYFPHF^DLSH GSAQVKGHGA KVAAALTKAV
 EHL^DLP^DGAL SELS^DLHAHK LRVD^DPNFKL LSHSLVTLA SHLPS^DFTPA VHASL^DKFLA NVSTVLTSKY R

β -chain

MLTAEKAAV TAFWGVK^DV^D EVGGEALGRL LVVYPWTQRF FESFG^DLSTA DAVMNNPKVK AHGK^DV^DLSF
 SNGMKHL^DDL KGTFALSEL HCD^DKLH^DV^DPE NFKLLGNVLV VVLARNFGKE FTPVLQAD^DFQ KVVAGVANAL AHRYH

22

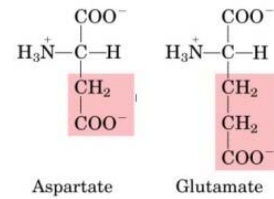
Origin of Asp-X sequence selectivity?



General mechanism of peptide bond hydrolysis

1: Interaction with the metal ion polarizes C=O bond

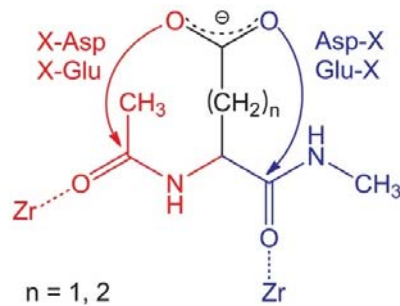
2: Attack of a nucleophile (H_2O , -OH , -O^-) to C is required



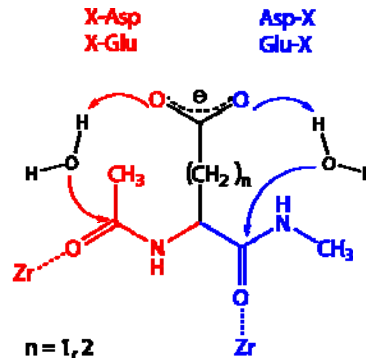
Aspartate

Glutamate

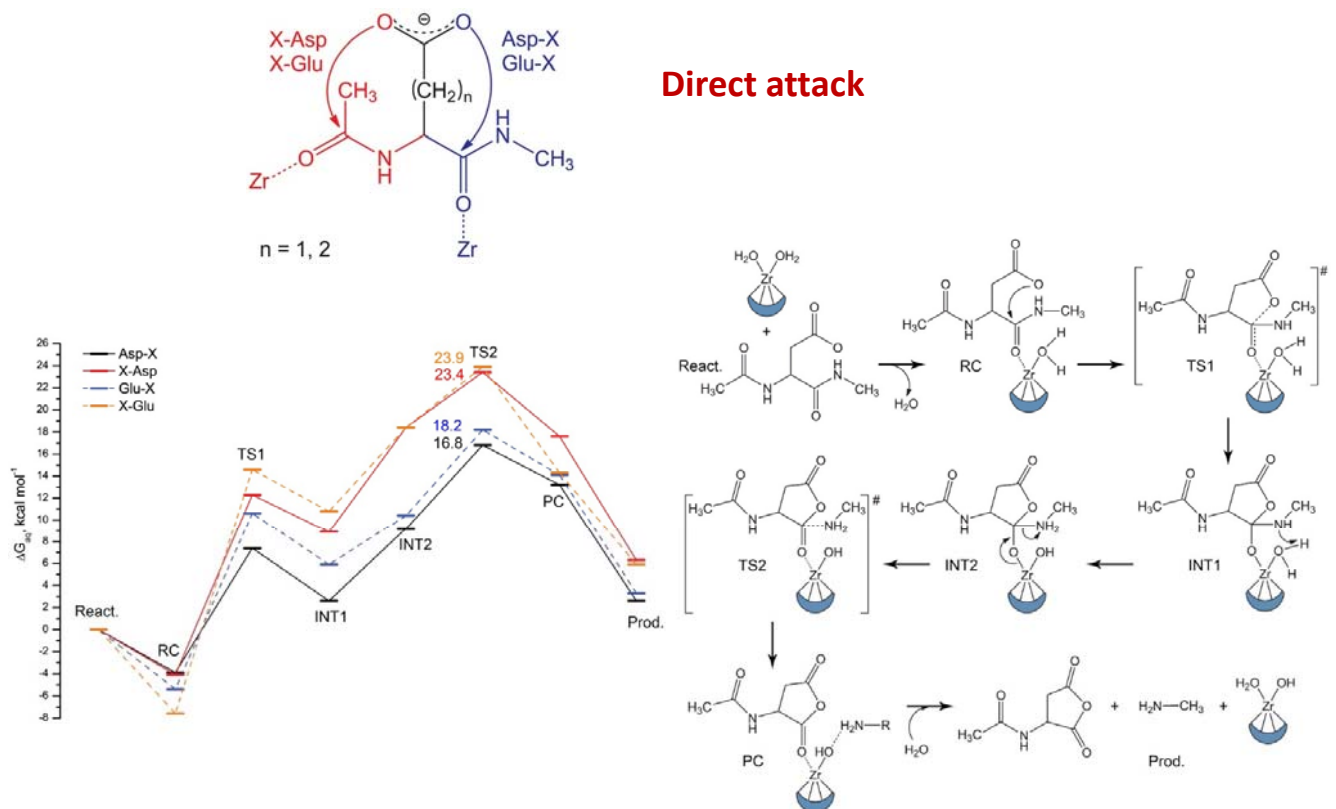
What is the actual nucleophile?



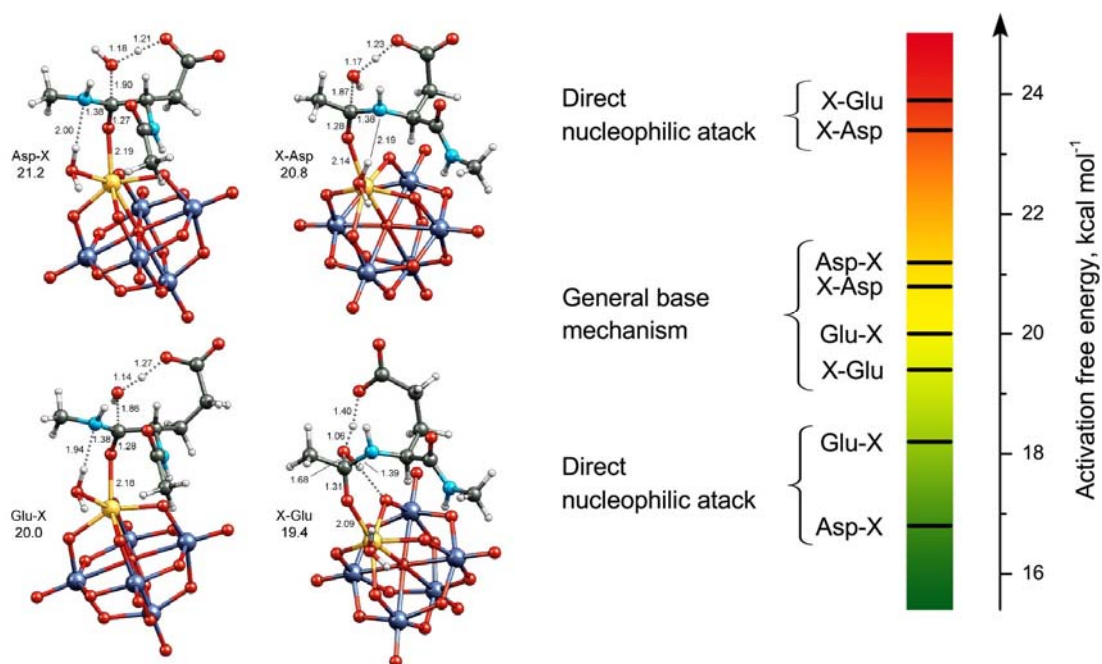
Direct attack



General Base

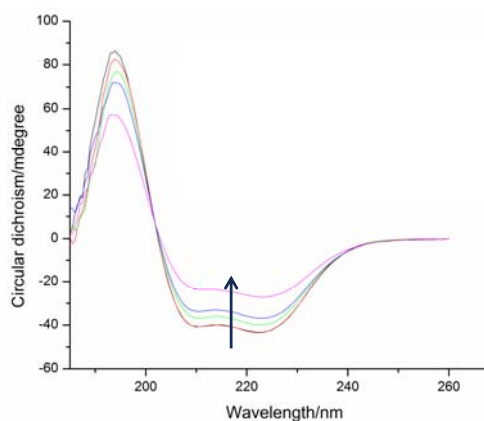


General base mechanism



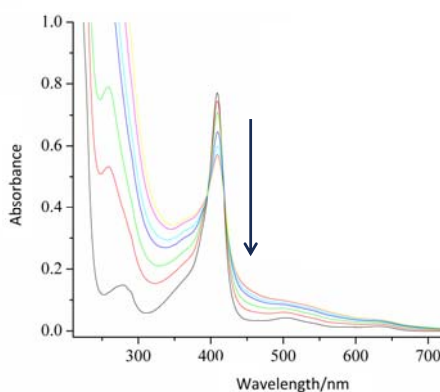
How do Zr-POMs and Proteins interact?

CD Spectroscopy



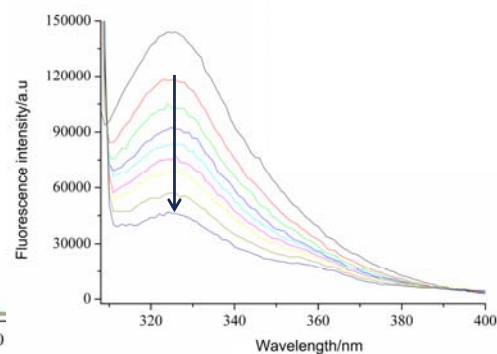
34% Decrease in α -helical content of myoglobin in the presence of 2:2 Zr-Keggin POM

Uv-Vis spectroscopy



No shift in Soret band
Decrease in intensity

Trp fluorescence spectroscopy

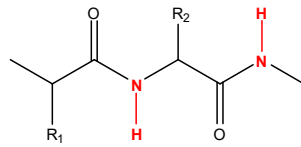


$$\log \frac{(F_0 - F)}{F} = \log K_q + n \log [Q]$$

$$K_q = 8.5 \times 10^4 \text{ M}^{-1}$$

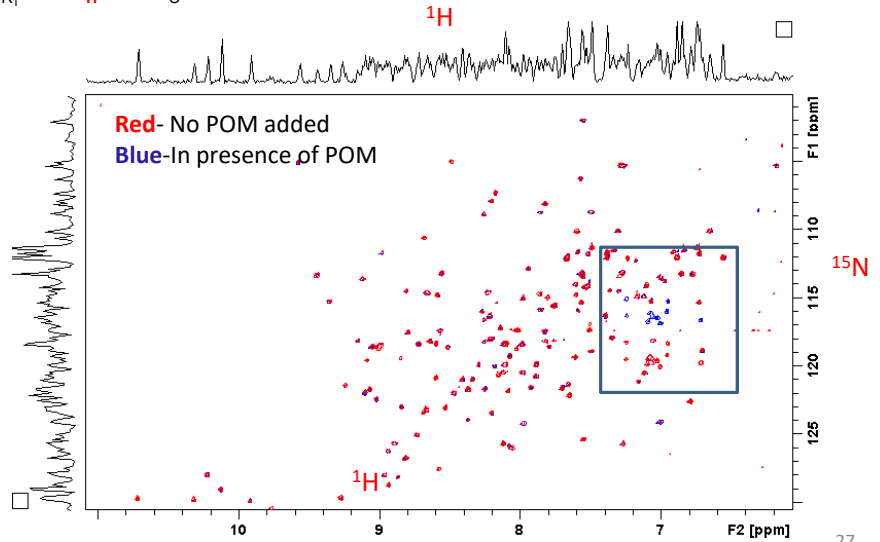
POM and protein interact, but protein remains largely folded upon POM binding

¹⁵N-¹H heteronuclear single quantum coherence (HSQC) NMR spectroscopy



¹⁵N I=1/2, 0.34% natural abundance

- Coupling between amide N and amide H.
- Each amino acid (except for proline) is characterized by at least one N-H cross peak.
- ¹⁵N-¹H HSQC = fingerprint of a protein
- ¹⁵N-¹H HSQC peaks are sensitive to changes in the chemical environment: excellent tool for probing interactions.

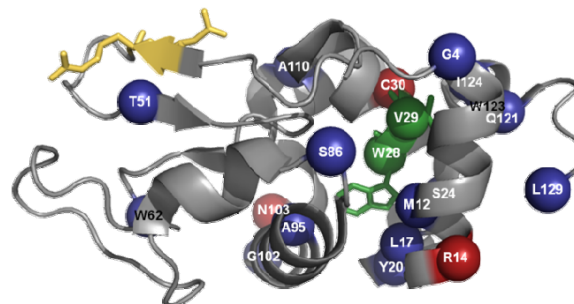
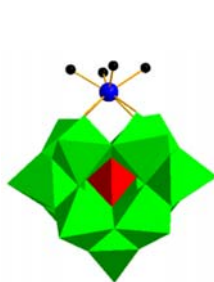
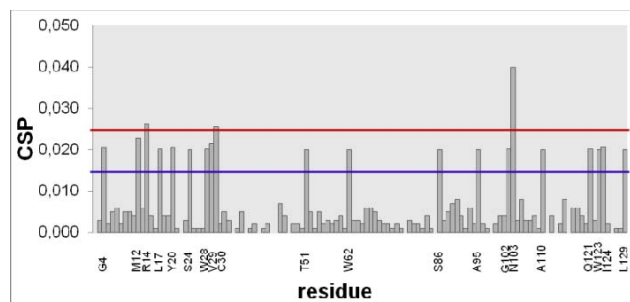


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¹H, ¹⁵N-HSQC NMR of Lysozyme in the presence of POM

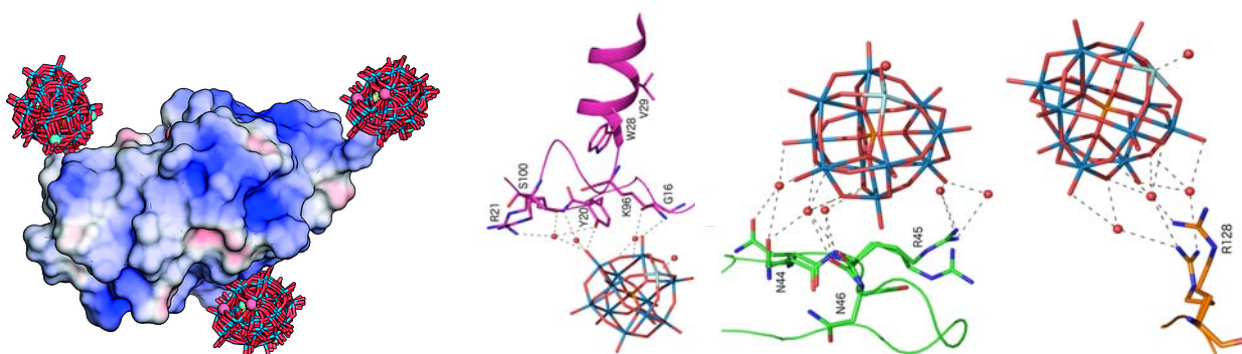
Chemical Shift Perturbation

$$\sqrt{(\Delta\delta H)^2 + \frac{(\Delta\delta N)^2}{25}}$$



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Single crystal X-ray structure of a non-covalent complex between Zr-POM and Lysozyme

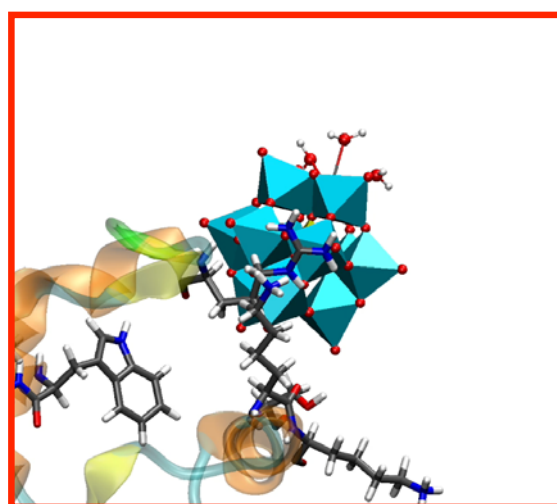
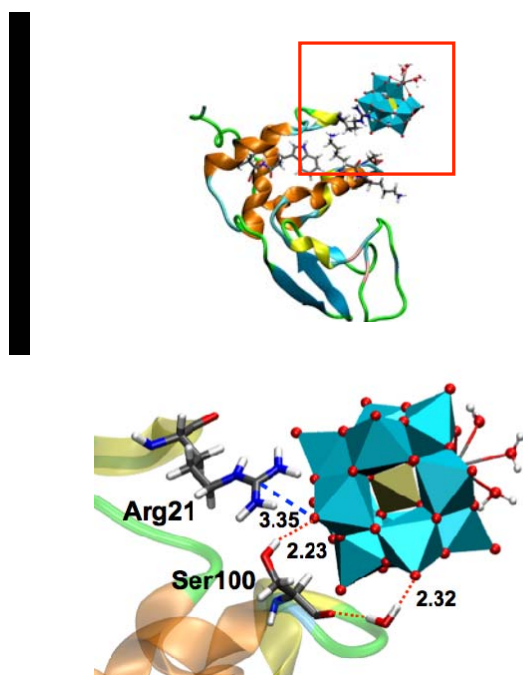


- Binding sites are at the positively charged regions of the protein
- Binding occurs via water-mediated H-bonding and electrostatic interactions

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Molecular Dynamics Simulations

Typical 20 ns run:



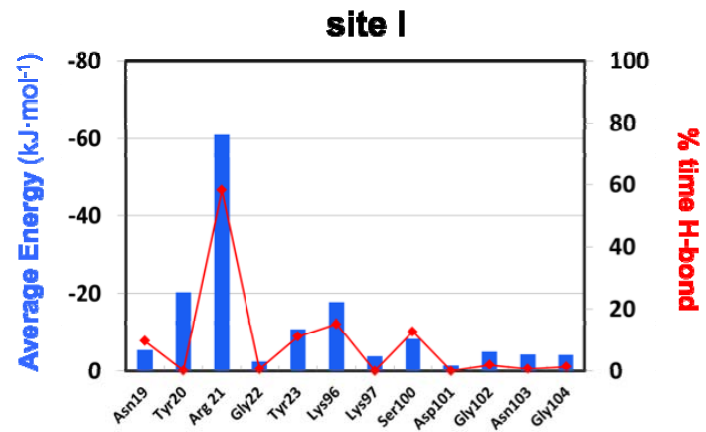
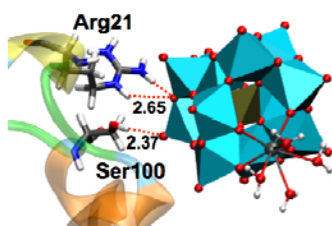
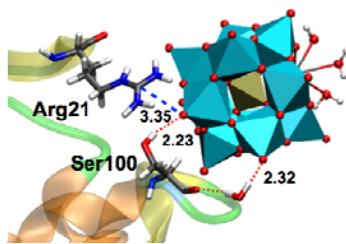
POM on protein surface:

- **electrostatic** (Arg21, Lys96, Lys97)
- **H-bonds** (Arg21, Lys96, Lys97, Ser100, Tyr23...)
- water-mediated

4

MD Analysis of amino acid specific interaction:

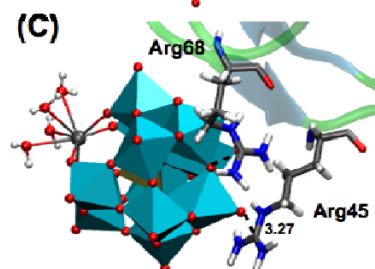
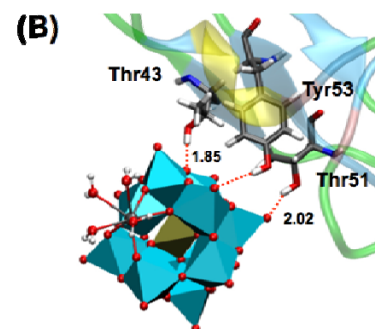
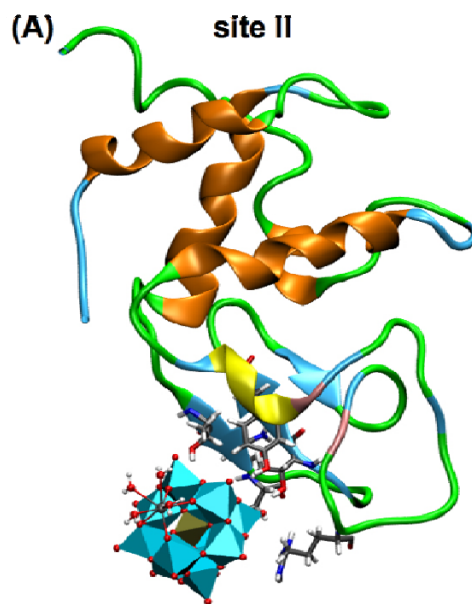
Site I



Averaged values over 100 ns sampling.

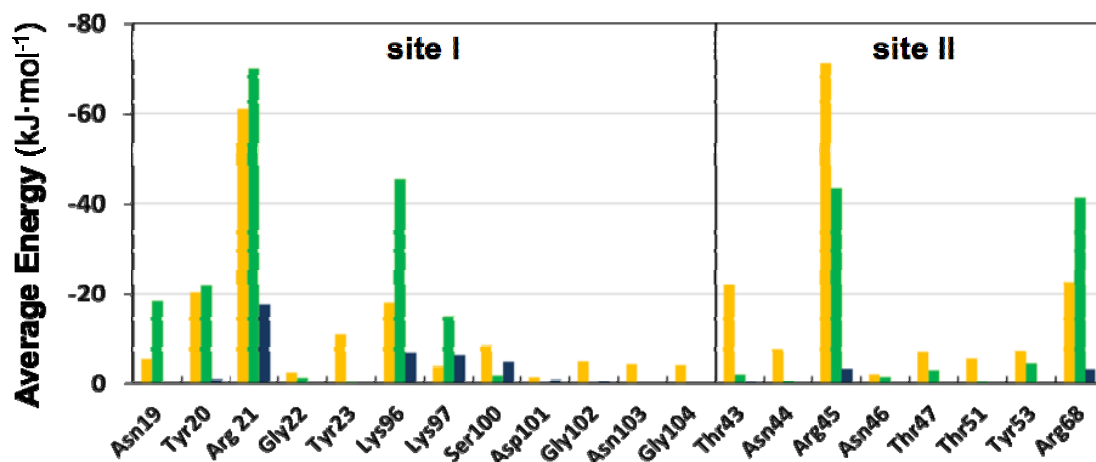
5

Site II

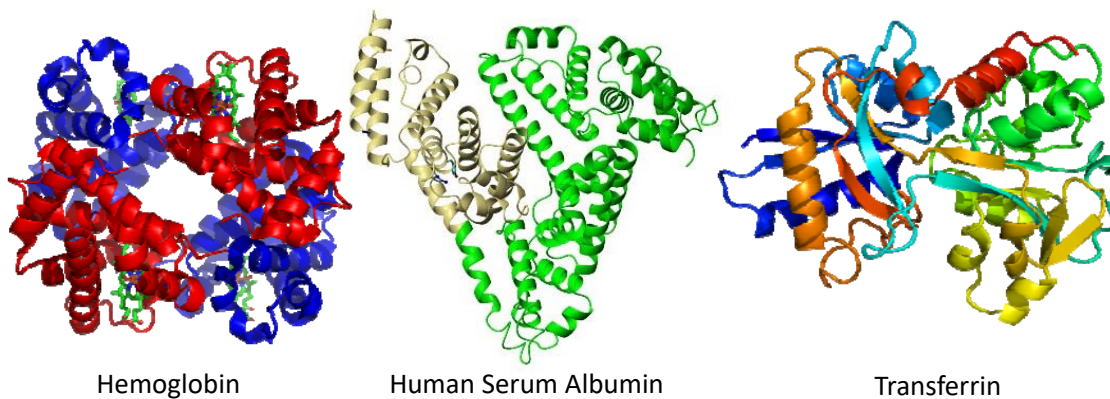
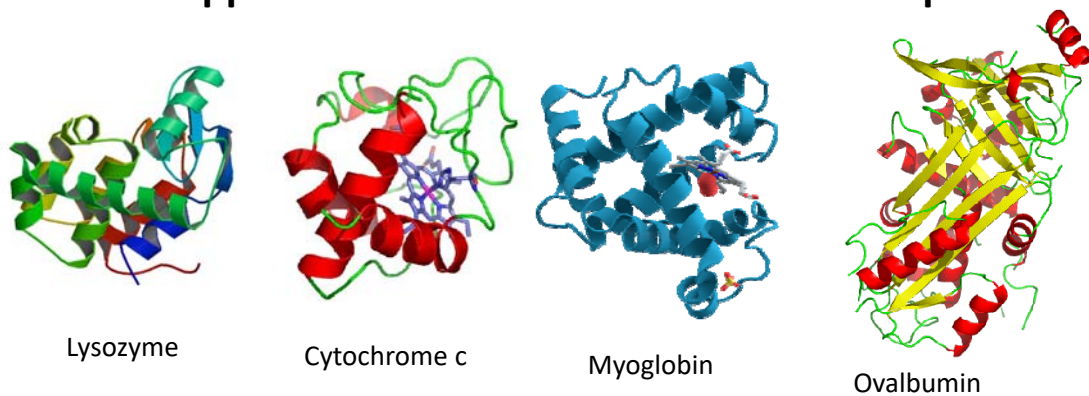


Amino acid individual analysis:

preferential interaction with positively charged amino acids (Lys, Arg)

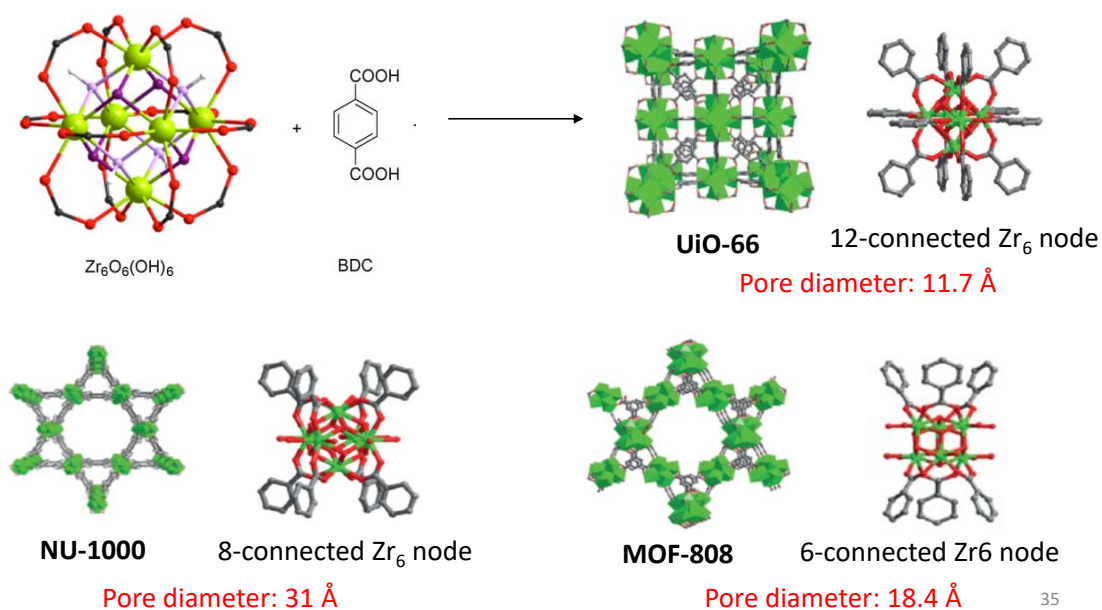


Method is applicable to other POMs and different proteins



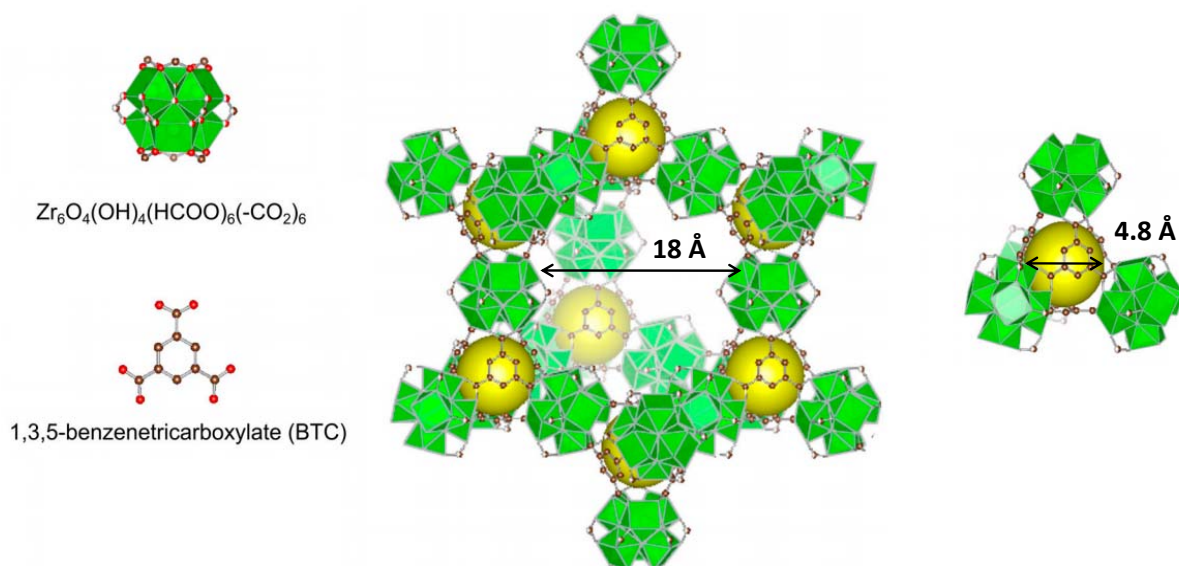
Nanozymes: materials with enzyme-like properties

- Metal–organic frameworks (MOFs) based on $\{Zr_6O_6\}$ clusters as potential nanozymes
- Catalytic activity of MOFs towards biomolecules has been very scarcely explored

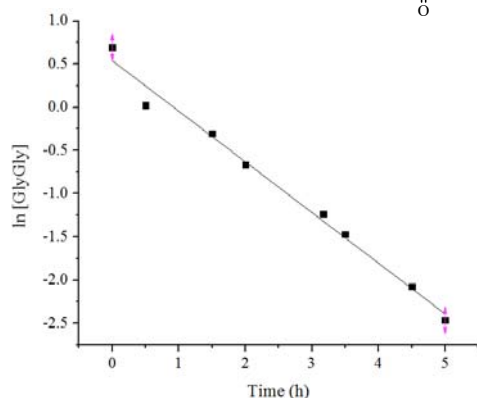
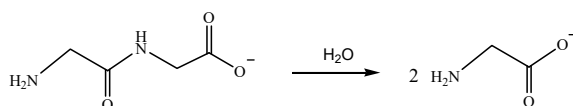


MOF-808

6-connected Zr_6 node: $Zr_6O_4(OH)_4[C_6H_3(CO_2)_3]_2(HCOO)_6$
Pore diameter: 18 Å, 4.8 Å



Hydrolysis of Gly-Gly by MOF-808

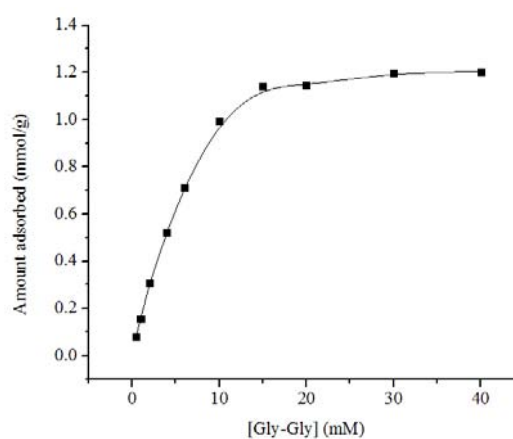
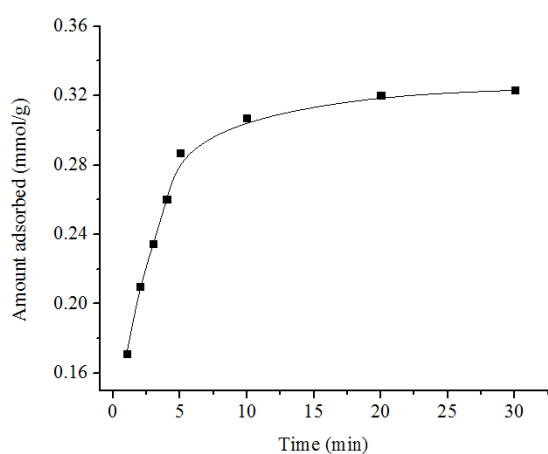


Catalyst	Experimental condition	$t_{1/2}$
MOF-808	pD 7.4, 60 °C	1.19 h
2:2 Zr-K POM	pD 7.4, 60 °C	29 d

$$k_{\text{obs}} = 1.62 \times 10^{-4} \text{ (s}^{-1}\text{)}$$
$$t_{1/2} = 1.19 \text{ h}$$

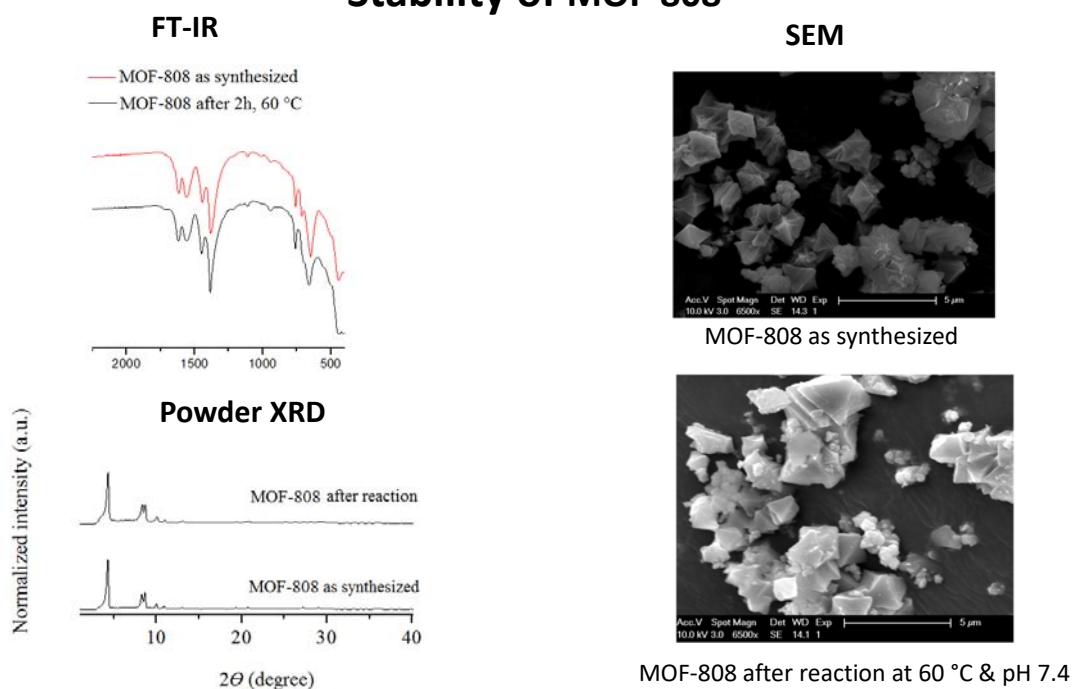
Rate enhancement of several orders of magnitude compared to uncatalyzed reaction!

Adsorption of Gly-Gly on MOF-808



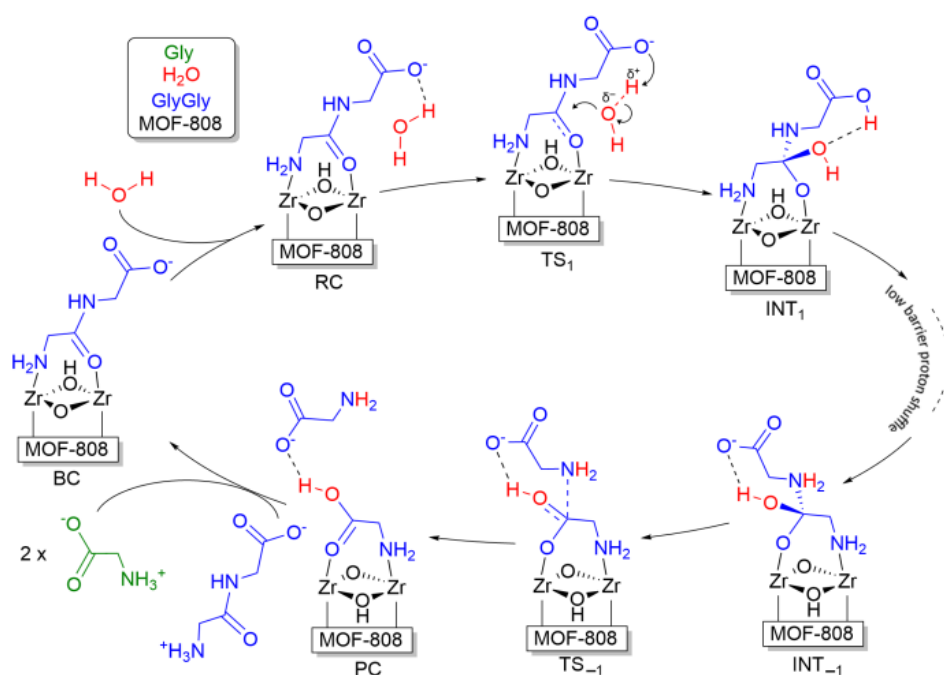
- Adsorption of Gly-Gly was at equilibrium after 20 min at RT.
- A saturation adsorption capacity of 1.15 mmol of Gly-Gly per 1.0 g of MOF-808 (0.65 mmol of Zr_6O_8 cluster) was obtained.

Stability of MOF-808



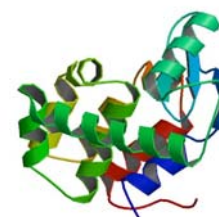
- The structure of MOF is preserved during and after peptide hydrolysis
- The MOF can be recycled and reused several times without loss of activity

Two Zr(IV) are better than one!

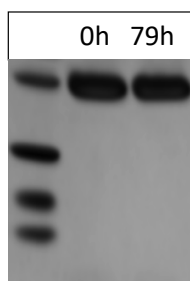


Can proteins be hydrolyzed by Zr-MOF?

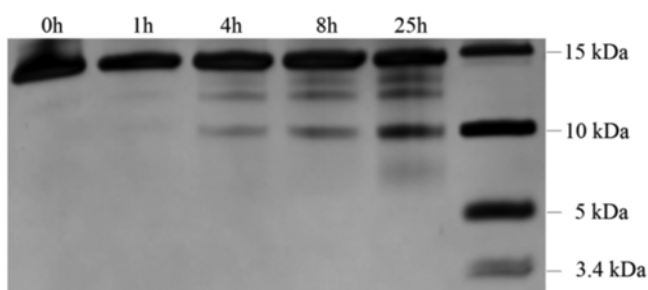
Henn egg white lysozyme (HEWL) 134 amino acids



HEWL pH=7.4



HEWL + MOF-808 pH=7.4



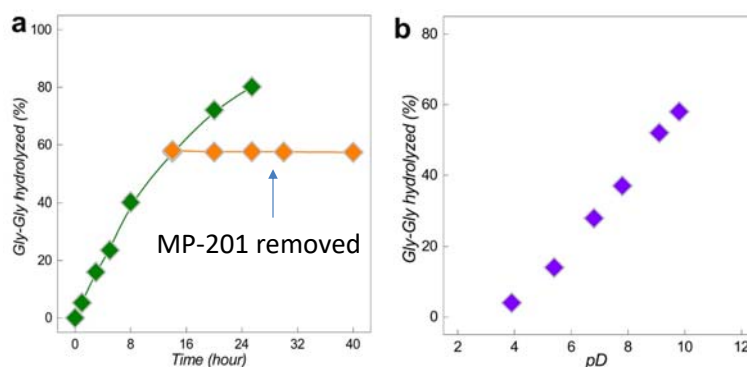
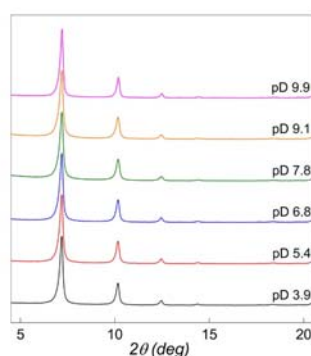
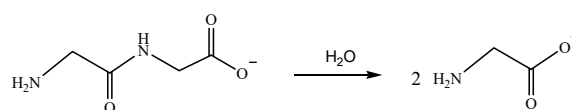
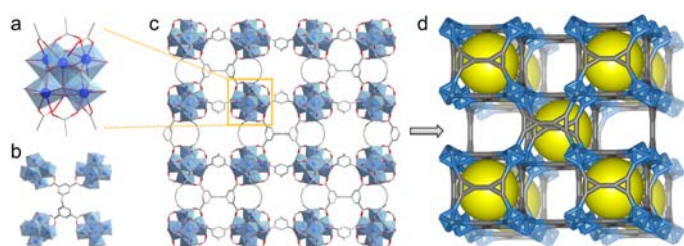
Hydrolysis at:

- Asp 119 (13.1 kDa fragment)
- Asp18 (12.5 kDa fragment)
- Asp18 and Asp119 (10.3 kDa)
- Asp52 (8.5 kDa)



H. G. T. Ly, et al.
J. Am. Chem. Soc. **2018**, 140, 6325

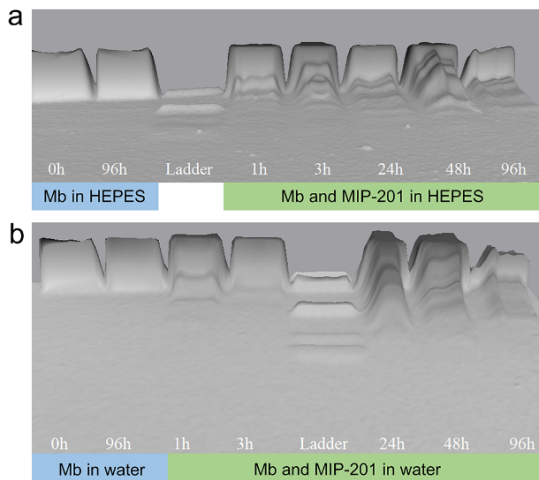
MIP-201: an extremely stable nanozyme with protease activity



Peptide hydrolysis occurs both on the surface of MOF and inside the MOF pore

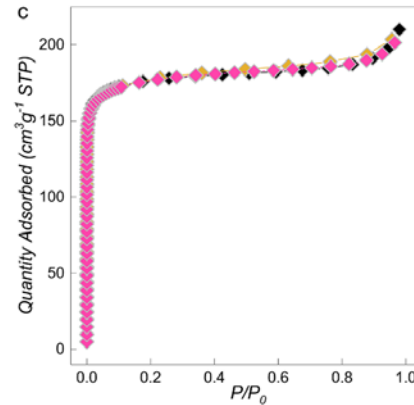
Nat. Commun. **2022**,13,1284.

Hydrolysis of Myoglobin by MIP-201



silver stained SDS-PAGE gel of Mb hydrolysis

Nat. Commun. 2022,13,1284.



Nitrogen adsorption isotherms of MIP-201 samples before (◆) and after the application in hydrolysis of Mb in HEPES buffer (◆) and water (◆).

MOF pores are not clogged after hydrolytic reaction

Where does protein hydrolysis occur?

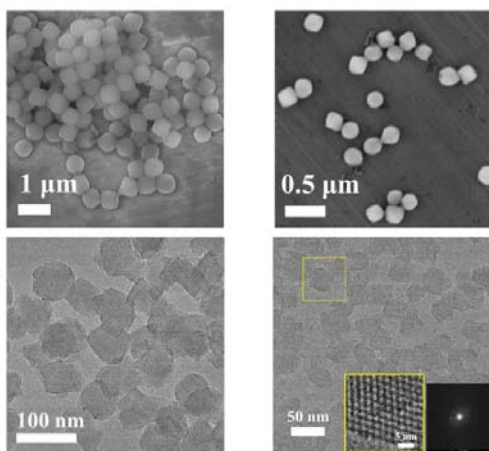
Development of MOF-808 nanozymes having the same structural features but different crystal sizes (surface area)

MOF crystal size 35-850 nm

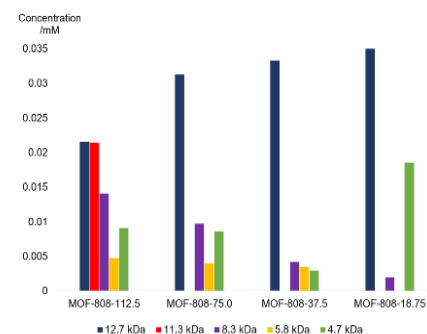
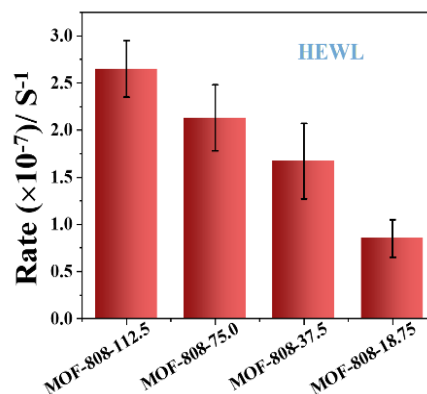
MOF surface area decreases



Reactivity decreases
Number and yield of peptide fragments changes



Chem. Mater., 2021, 33, 7057-7066.

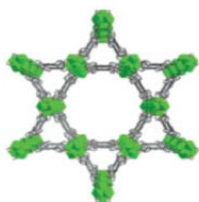


Catalytic activity is directly proportional to the external surface area of the MOF particles, suggesting that protein hydrolysis is likely to occur on the MOF surface.

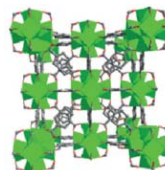
Other Zr/Hf based MOFs and metal-oxo clusters exhibit unique nanozymatic activity towards proteins



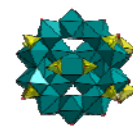
MOF-808



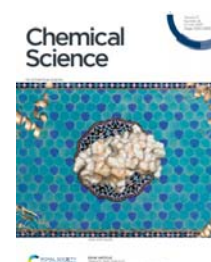
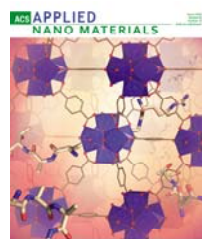
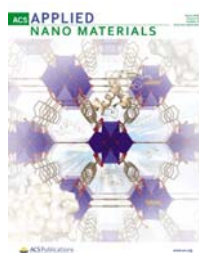
NU-1000



UiO-66



Discrete Hf₁₈ cluster



J. Am. Chem. Soc. **2018**, 140, 6325.
ACS Appl. Nano Mater. **2020**, 3, 8931.
Chemical Science **2020**, 11, 6662.

Angew. Chem. Int. Ed., **2020**, 132, 9179.
Chem. Mat. **2021**, 33, 7057.
ACS Appl. Nano Mater **2021**, 4, 5748.
Eur. J. Inorg. Chem. **2022**, e202200145

Nat. Commun. **2022**, 13, 1284.
Nanoscale, **2021**, 13, 12298.
Chem. Eur. J., **2021**, 27, 17230.
Mater. Adv. **2022**, 3, 2475.

Advantage of POMs and MOFs as Artificial Proteases

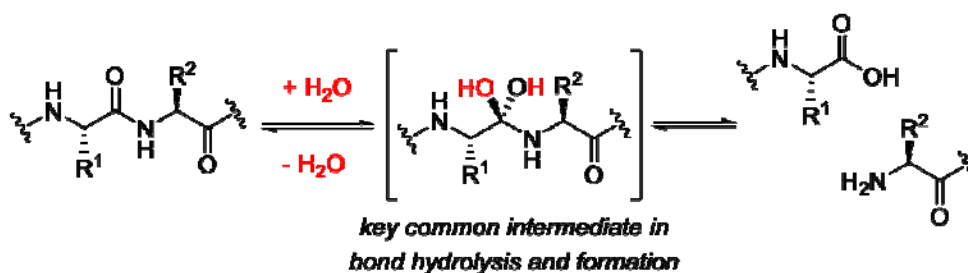
- Inexpensive
- pH and temperature stable
- Reusable
- Tunable reactivity
- Stable in the presence of surfactants →

Possibility to hydrolyze poorly soluble and insoluble proteins!

Can we use the same catalysts to make peptide bonds instead of breaking them?

Direct peptide formation is highly desired, but challenging transformation

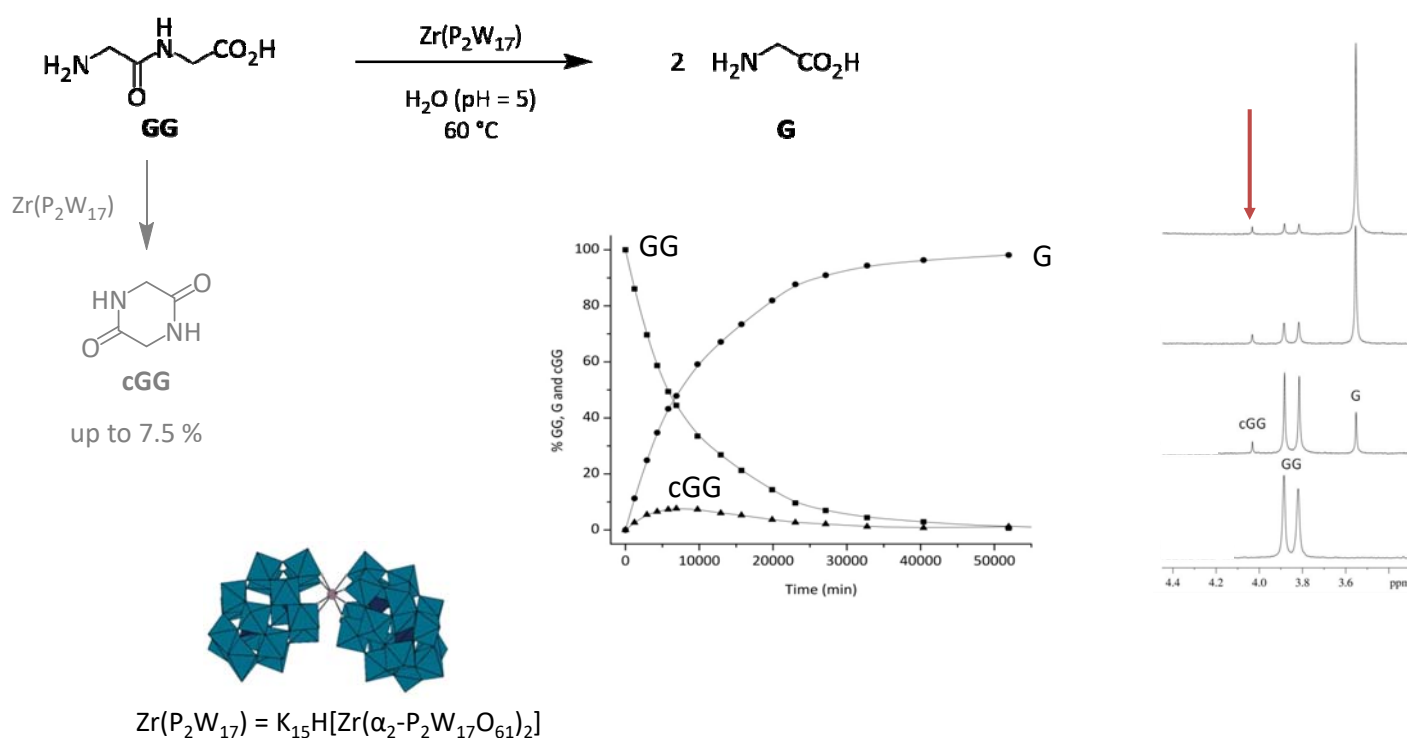
Hydrolysis and **Formation** of Peptides Are Two Lanes of the Same Road



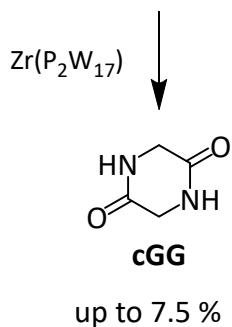
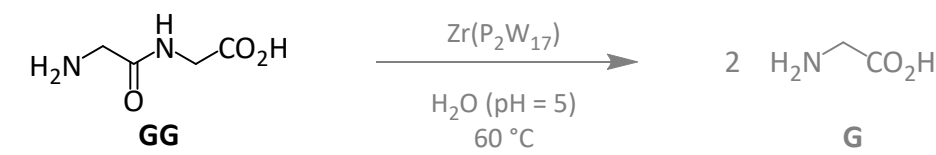
Principle of Microscopic Reversibility:
Same Intermediates Lead to Amide Bond Formation

47

Peptide bond also forms during peptide hydrolysis!



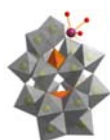
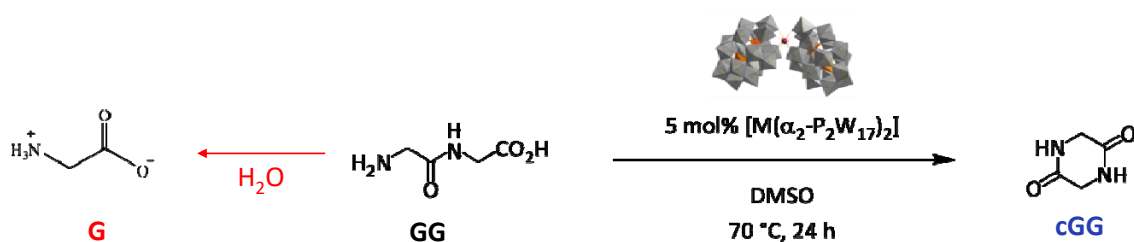
Can this equilibrium be re-directed?



Potential advantages

- High catalytic activity in the presence of water
- Tolerance to polar functional groups (CO₂H, NH₂...)
- Non-activation of carboxylic acid or amine groups
- Catalyst recycle

Zr^{IV} / Hf^{IV}-POMs are unique catalysts for making or breaking peptide bond



M($\alpha_2\text{-P}_2\text{W}_{17}$)

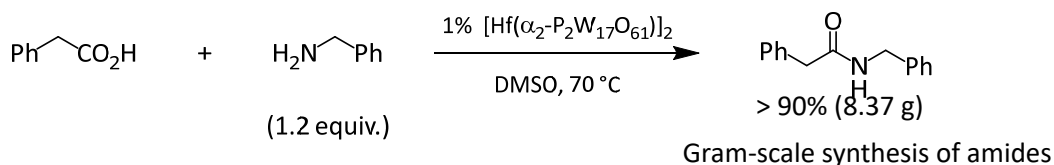
M = Fe, Cu, Mn, Co

M	Yield cGG (%)	Conversion (%)
Zr	36	95
Hf	75	75
Ce ^{III}	0	—
Ce ^{IV}	0	—

Solvent	G	cGG
H ₂ O	>99%	—
DMSO	—	>99%

- When M = Fe, Cu, Mn, Co no product was observed

Is intermolecular amide coupling possible?



Streamlined *amide bond formation*

- Low catalyst loading
- *No water scavengers*
- *No dry solvents*
- No chromatography

Robust *POM catalysis*

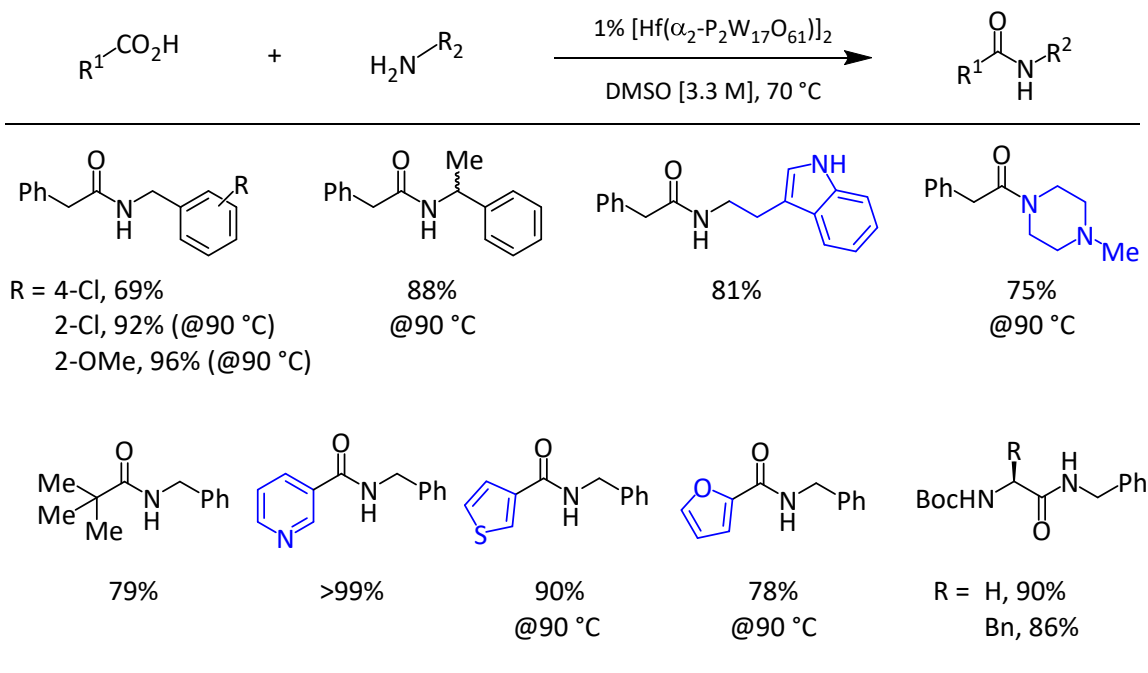
- Readily available
- *High water tolerance* (>220 molar excess)
- Recyclable



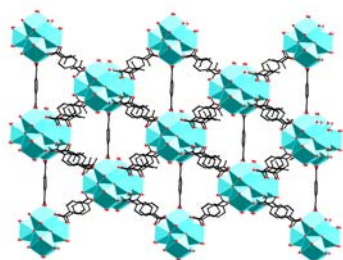
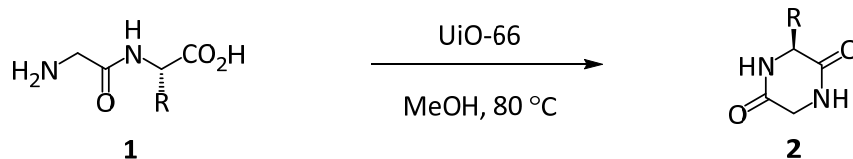
F. de Azambuja, T. N. Parac-Vogt, *ACS Catalysis*, **2019**, 9, 10245

F. de Azambuja, J. Lenie, T. N. Parac-Vogt. *ACS Catalysis*, **2021**, 11, 271.

Reaction scope tolerates various functional groups



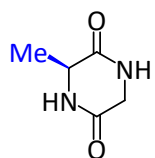
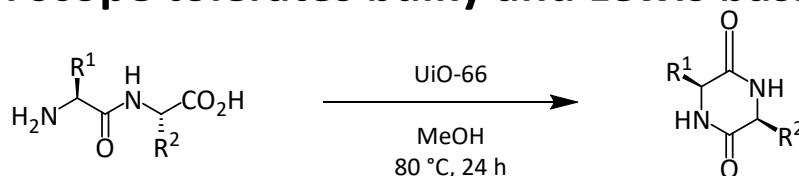
En route to heterogenous peptide bond formation: Zr-UiO-66 MOF as a heterogenous catalyst



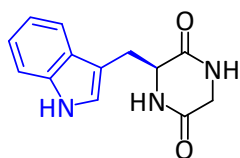
UiO-66

- MeOH, EtOH >> 2-PrOH, DMSO
- No peptide bond hydrolysis observed
- **No inert atmosphere, dry solvents or drying agents needed**

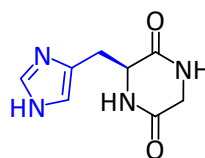
Reaction scope tolerates bulky and Lewis basic groups



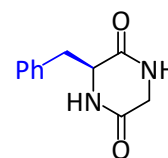
Gly-Ala: 96%
Ala-Gly: 87%



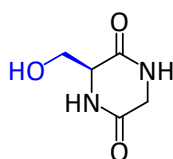
81%



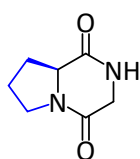
68%



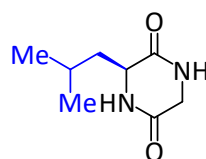
88%



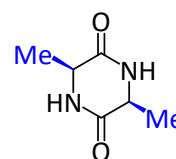
Gly-Ser: 81%
Ser-Gly: 40%



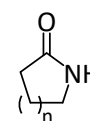
Gly-Pro: 82%
Pro-Gly: 79%



90%



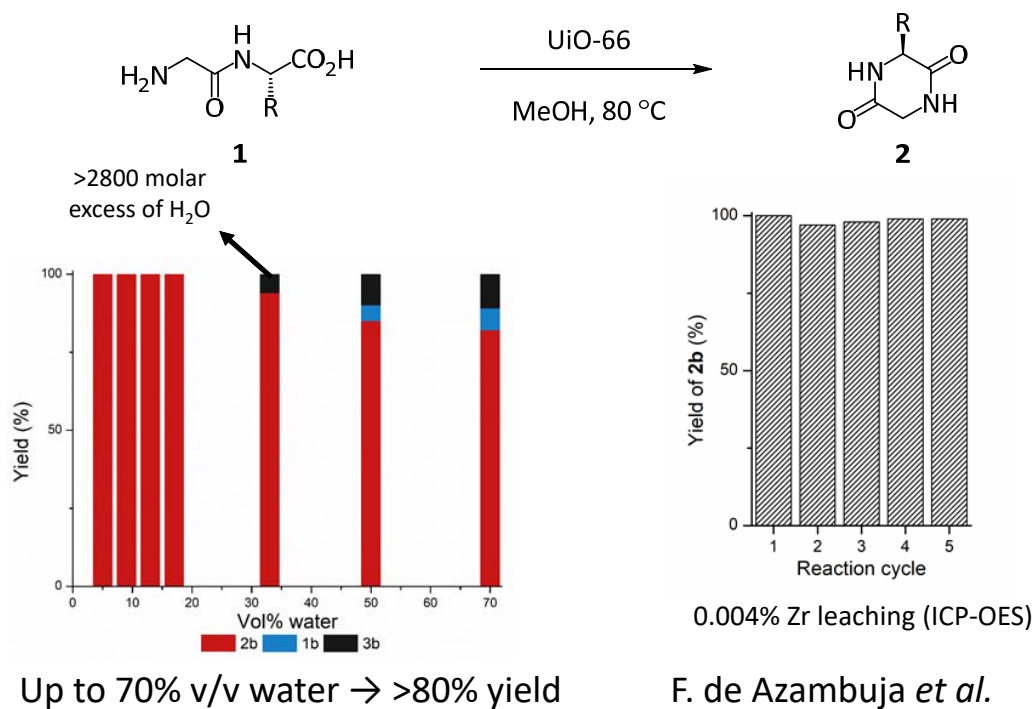
18%



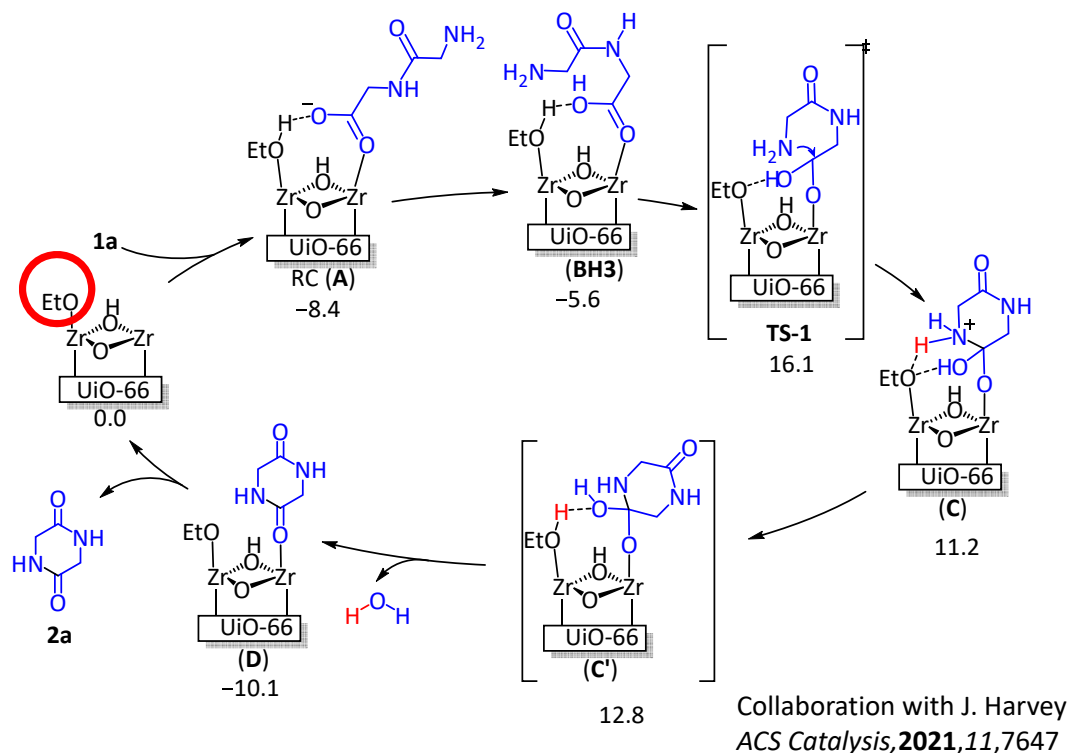
n = 1, 97%^a
n = 2, 98%^a

Isolated yields; no epimerization detected. ^a NMR yields.

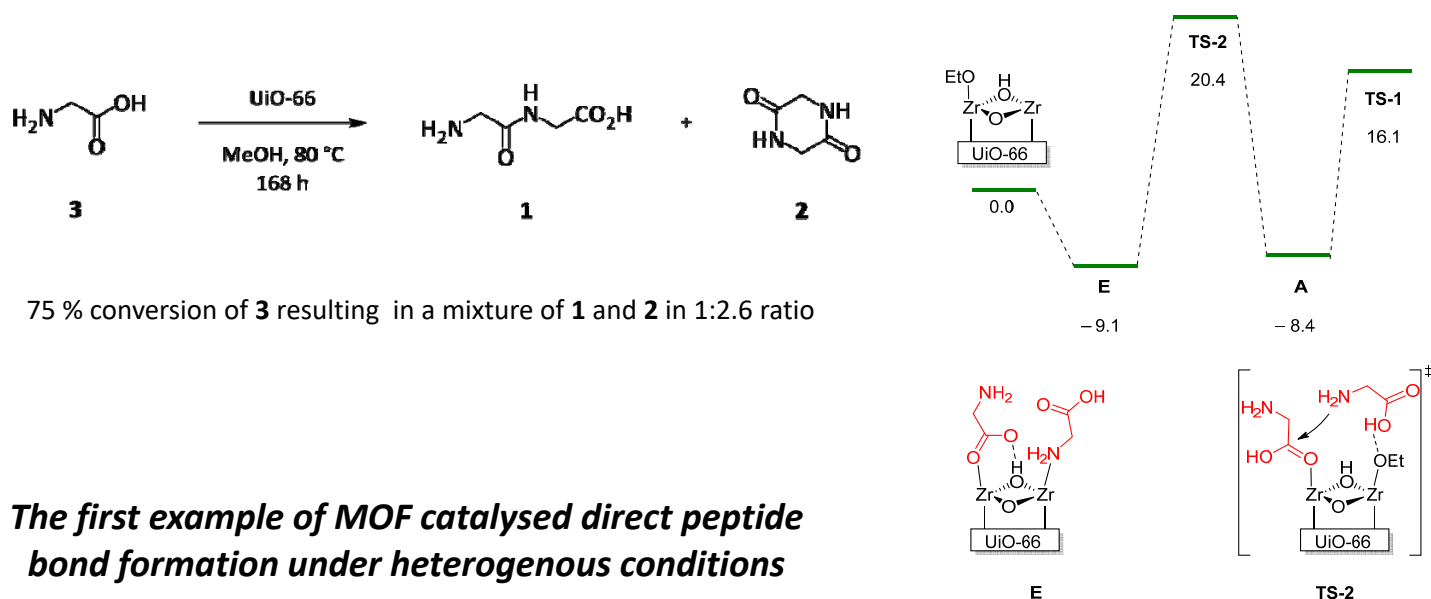
UiO-66 robustness → H₂O tolerance and recyclability



EtO group streamlines key proton transfers



UiO-66 affords also *intermolecular* peptide bond formation



75 % conversion of **3** resulting in a mixture of **1** and **2** in 1:2.6 ratio

F. de Azambuja *et al.* *ACS Catalysis*, **2021**, *11*, 7647

Conclusions

- Conceptually new approach towards achieving **selective** hydrolysis of proteins has been achieved by **combining the enzyme-like molecular recognition ability of POM scaffold** with the hydrolytic activity of a strong **Lewis acid metal cation** imbedded into the POM structure
- Hydrolysis occurs at near to **physiological pH**, with large **rate enhancement** compared to uncatalyzed reaction
- **Remarkable selectivity** toward hydrolysis of peptide bonds next to Asp-X residues
- The method is **versatile** and applicable to a **range of proteins** differing in size, charge and solubility
- First proof of principle of **heterogeneous catalysis of peptide bond hydrolysis by Zr-MOFs** has been demonstrated
- By applying the principles of **microscopic reversibility**, POMs and MOFs have been developed as **water tolerant catalysts** for **direct amide bond formation**
- The first example of **direct peptide bond formation under heterogenous conditions using MOF catalyst** has been achieved

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<https://lbc.chem.kuleuven.be/>