Electronic Supplementary Material (ESI) for Nanoscale Advances.

Hybrid chemo-enzymatic heterogeneous catalyst prepared in one step from zeolite nanocrystals and enzyme-polyelectrolyte complexes

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Supporting Information

1. Study and calculations of the charge ratio in the EPCs

Formation of the EPCs

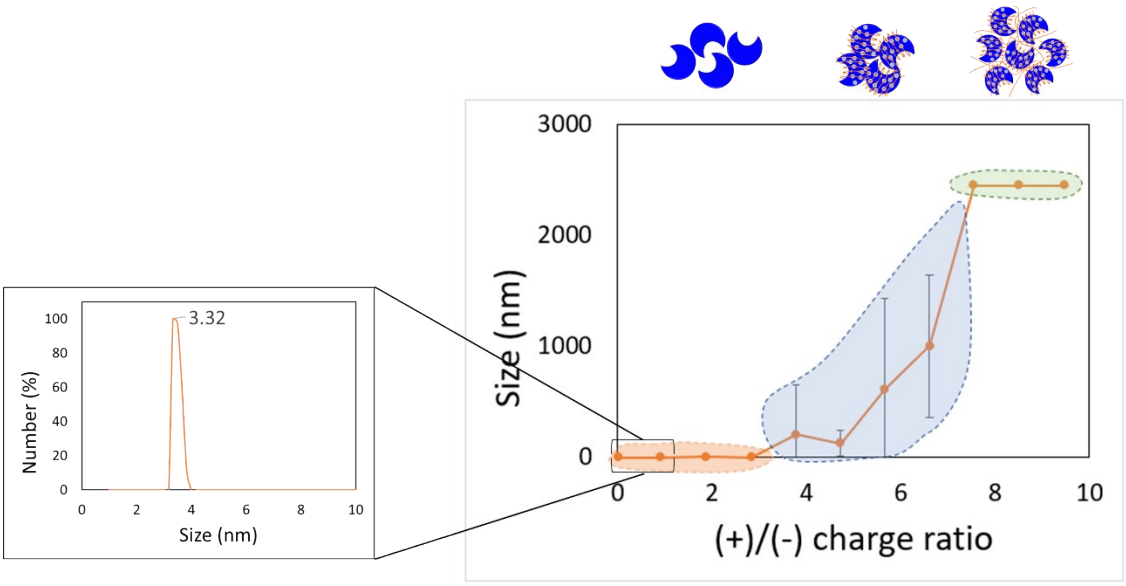

Figure S1. Measurements of the different size of the GOx-PAH complexes depending on the charge ratio. The measurements were performed by dynamic light scattering, on a solution of GOx (26.32 mg/ml) with increasing amount of a solution of PAH (4.22 mg/ml) and each measurement was repeated 10 times. The curve can be divided into 3 regions. In the first region (orange), GOx is mainly free in the solution and very small complexes are formed. The second region (blue) is a transition region where the size of the complexes is increasing with the charge ratio and thus the amount of PAH added. In the last region (green), the complexes are big enough to precipitate. The selected ratio is (+)/(-)=6.6 as it appears that no more free GOx was detected in solution. Note that EPCs are polydisperse in size (as reflected by the relatively large error bars in DLS measurements).

Charge ratio calculations

The charge ratio is calculated as followed:

PAH charge calculations

Molar mass = 17 500 $g.mol^{-1}$

Based on the structure of PAH, the molar mass of one monomer unit can be calculated (=93.5 $g.mol^{-1}$) and the number of units in one molecule of PAH is calculated as follow:

$$N = \frac{Total\ M_w}{M_w of\ one\ unit} = 187.16\ units \qquad (1)$$

Each unit carries a positive charge from the NH_3^+ group and therefore, each molecule of PAH bear **187.16** positive charges. The number of positive charges/mol is obtained by:

$$\left[\frac{(+)charges}{mol}\right] = 187.16\frac{(+)charges}{molecule} * N_A = 1.12 * 10^{26} \qquad (2)$$

GOx calculations

Molecular weight = 160 000 Da

The number of charges carried by each GOx molecule depends on the pH. These values are found on PDB2PQR (Code: 1CF3, GLUCOSE OXIDASE FROM APERGILLUS NIGER). At pH 6.0, each subunit carries 14.52 negative charges. The number of negative charges/mol is calculated following Equation 2.

(+)/(-) charge ratio

Depending on the concentration of each solution (PAH and GOx), the numbers of positive and negatives charges are calculated.

2. Study of the inorganic catalyst (TS-1 and Aer_TS-1)

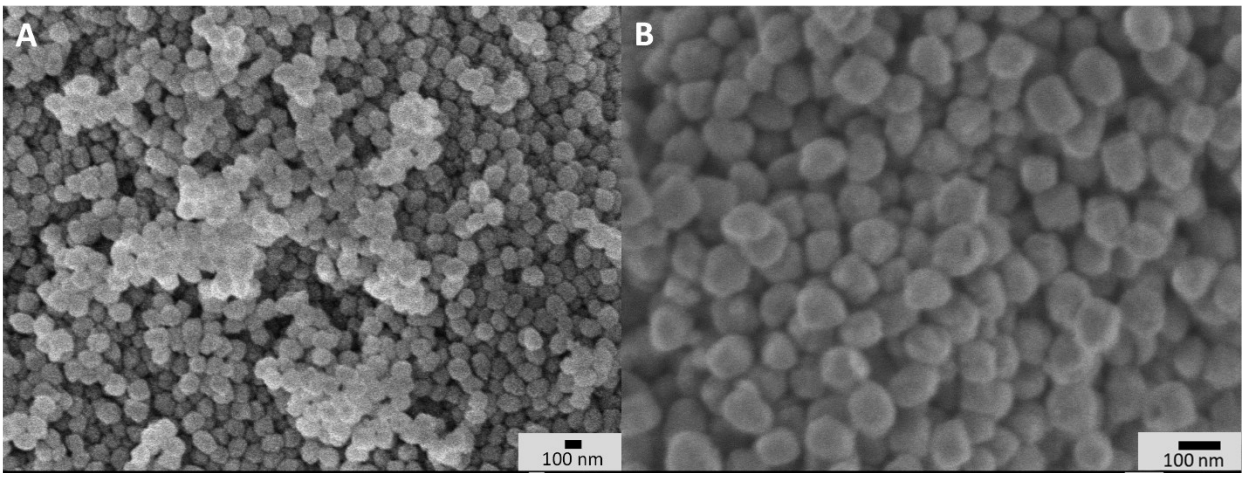

Figure S2. A) SEM images of TS-1 nanocrystals. B) SEM images of Hybrid_EPCs. Experimental conditions: powders were metallized with chromium. Image A) was measured at 5.0 kV and image B) was measured at 1.0 kV

3. Study of the organic catalyst in its different forms (GOx, EPCs, Hybrid_GOx and Hybrid_EPCs)

Stability toward pH and thermal variations

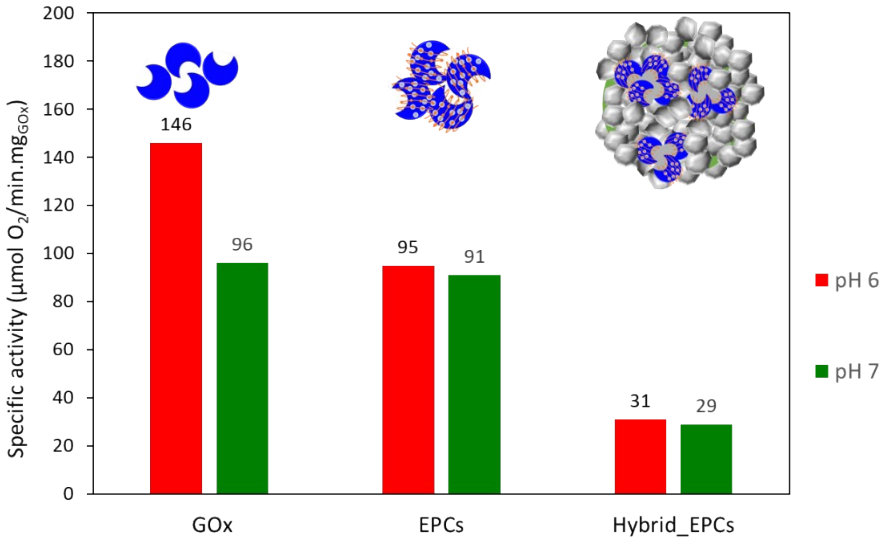

Figure S3. Comparison of the specific activity of the samples **GOx**, **EPCs**, and **Hybrid_EPCs** at pH 6 and pH 7. This figure highlights the stabilization effect toward pH variation brought by the formation of EPCs. Experimental conditions: T = 45°C, [Glucose] = 200mM

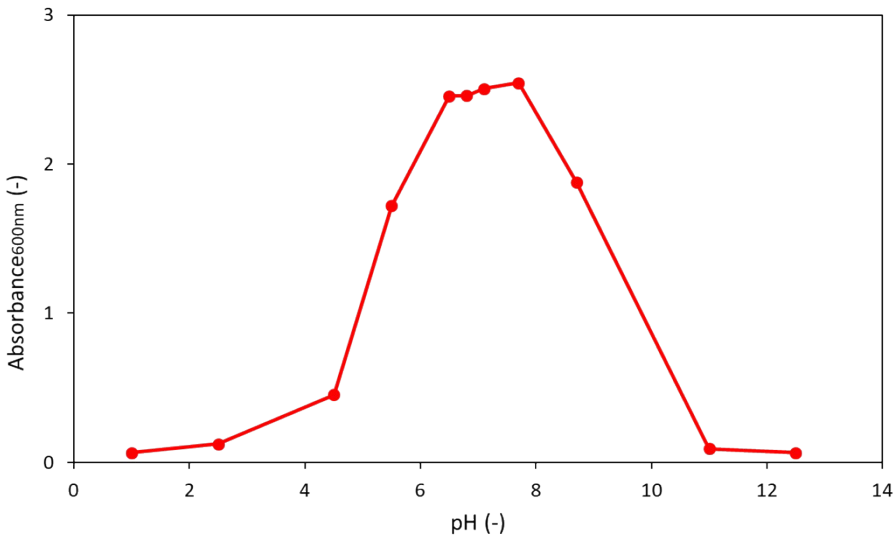

Figure S4. Study of the pH range in which the **EPCs** are stable. The absorbance of an EPCs suspension was measured at 600 nm at different pH. It shows that the EPCs are stable in a pH range between 5.8 and 8.

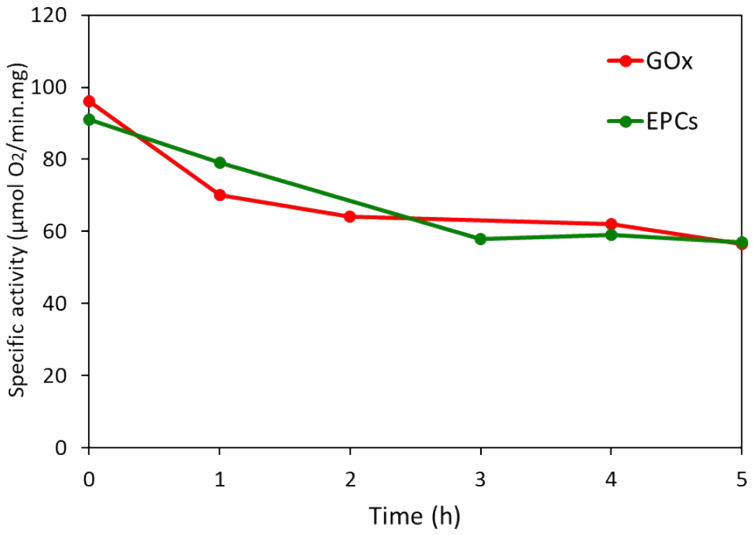

Figure S5. Stability of **GOx** and **EPCs** toward thermal denaturation. Experimental conditions: A solution of GOx and a suspension of EPCs (15.48 $mg_{GOx}.mL^{-1}$) were placed in a 45°C hot bath. The enzymatic activity was tested at regular interval of time to see the influence of thermal denaturation.

GOx loading

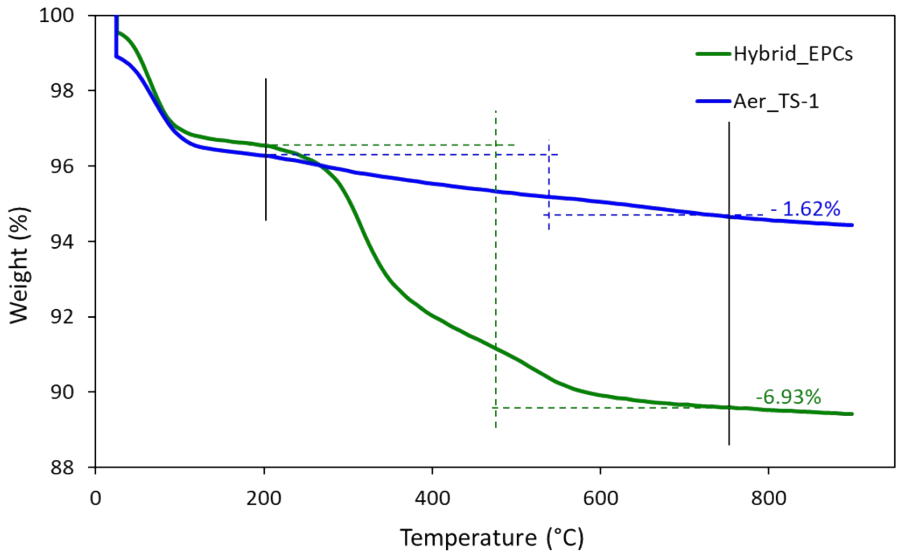

Figure S6. TGA of **Hybrid_EPCs** and **Aer_TS-1** used to determine the experimental loading of the organic content in the hybrid catalyst. The experimental loading of organic matter in **Hybrid_EPC** is calculated as followed:

$$Organic\ matter\ (\%) = \%\ Mass\ loss\ Hybrid_{EPCs 200°C\ to\ 750°C} - \%\ Mass\ loss\ Aer_{TS-1\ 200°C\ to\ 750°C} = 5.31\%$$

The theoretical loading amounts to 50 $mg.g^{-1}$ (45 mg of GOx and 5 mg of PAH).

4. Procedure for the catalytic chemoenzymatic cascade

Analytics

The concentrations different compounds found in the reaction medium were computed through different analytical methods described in the experimental section.

The formulas used to define the reaction performance are showed below as the conversion toward a reagent, the yield and selectivity toward a product.

$$Conversion_{Reagent(t)}(\%) = 100 * \frac{C_{Reagent(t=0)} - C_{Reagent(t)}}{C_{Reagent(t=0)}}$$

$$Yield_{Product(t)}(\%) = 100 * \frac{C_{Product(t)}}{C_{Reagent(t=0)}}$$

$$Selectivity_{Product(t)}(\%) = 100 * \frac{C_{Product(t)}}{\sum C_{Products(t)}}$$

Cascade reaction repetition

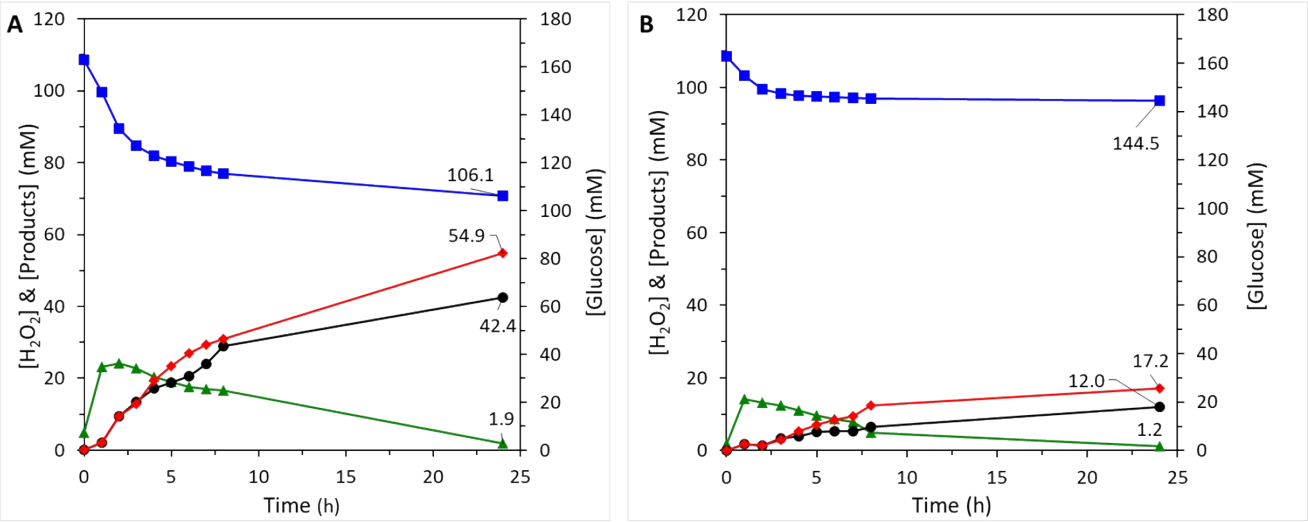

Figure S7. A) Repetition of the one-pot chemo enzymatic epoxidation of allyl alcohol with Hybrid_EPCs. Experimental conditions: T=45°C, [allyl alcohol] = 0.9 M, [Glucose] = 0.16 M, [Hybrid_EPCs] = 5g.L^{-1}. The repetition of the cascade reaction test was performed with a new batch of hybrid catalyst following the same synthesis protocol. The performance was slightly lower in this second experiment, reaching an epoxide yield of 26% (compared to 30% during the first test) and a glucose conversion of 35%. The selectivity toward glycidol was improved with a

value of 77%. The leaching of GOx was measured by Bradford assay and amounts to 7%. B) One-pot chemo enzymatic epoxidation of allyl alcohol with the two separate catalytic species Aer_TS-1 and the PAH-GOx complexes. Experimental conditions: T=45°C, [allyl alcohol] = 0.9 M, [Glucose] = 0.16 M, [Aer_TS-1] = 5g.L^{-1}, [GOx-PAH complexes] = 0.048 g.L^{-1}. ▲ Hydrogen peroxide, ● glycidol, ■ Glucose, ♦ Total (Glycidol+Glycerol).