Supplementary information for the article

Composites based on Heparin and MIL-101(Fe): drug-releasing depot for anticoagulant therapy and advanced medical nanofabrication

Vladimir V. Vinogradov,[*,a] Andrey S. Drozdov,[a] Leila R. Mingabudinova,[a] Emiliya M. Shabanova,[a] Nina O. Kolchina,[a] Elizaveta I. Anastasova,[a] Alina A. Markova,[b] Alexander A. Shtil,[a,b] Valentin A. Milichko,[c] Galina L. Starova,[d] Rafaella L.M. Precker,[e] Alexandr V. Vinogradov,[a] Evamarie Hey-Hawkins,[e] and Evgeny A. Pidko, [*a,f]

[a]*Laboratory of Solution Chemistry of Advanced Materials and Technologies, ITMO University, Saint-Petersburg, 197101, Russian Federation.*
E-mail: pidko@scamt.ru, e.a.pidko@tudelft.nl, vinogradov@scamt.ru
[b]*A.N.Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, 119991, Russian Federation*
[c]*Department of Nanophotonics and Metamaterials, ITMO University, Saint-Petersburg, 192001, Russian Federation*
[d]*Department of Chemistry, Saint-Petersburg State University, Saint-Petersburg, 198504, Russian Federation*
[e]*Institute of Inorganic Chemistry, Leipzig University, Leipzig, D-04103, Germany*
[f]*Chemical Engineering department, Delft University of Technology, Delft, 2629 HZ, The Netherlands*

Experimental

Materials

Synthesis of MIL-101(Fe).

The MIL-101(Fe) was prepared as described in.28 In brief, a mixture of 0.3375 g of iron (III) chloride, $FeCl_3$*$6H_2O$ (97%), 0.103 g of terephthalic acid, C6H4-1,4-(CO2H)2 (97%), and 15 mL of N, N-dimethylformamide (DMF) was placed into a Teflon container inside the autoclave. The mixture was heated for 3h at 408K under static conditions. Once the synthesis was completed, the solid product was twice filtered off using two glass filters with a pore size 40-100 um to remove free terephthalic acid. Then a solvothermal treatment was sequentially performed using 95% EtOH with 5% H2O at 353K for 24 h and washed 3 times with distilled water at 90 ^{o}C for 2 hours. The solid was dried overnight and stored at 433K under air atmosphere.

Synthesis of boehmite (alumina).

An alumina sol was prepared as described in ref. 1S . Essentially, 2.28 g of Al (C3H7O)3 was added to 50 mL of deionized water at 80◦C and a white precipitate was formed immediately. Before ultrasound treatment the precipitate was kept at 80◦C under vigorous stirring for 15 min to complete the formation of boehmite NPs and to fully evaporate isopropanol formed during hydrolysis. The final suspension was sonicated for 2 h to obtain a viscous sol that was then cooled to room temperature. The mass fraction of alumina nanoparticles in the final sol was 2%.

Sorption of heparin on MIL-101(Fe) and MIL-101(Cr).

2 mg of MIL-101(Fe) of MIL-101(Cr) was added to a solution of 200 μL heparin (5000 U/mL) in 2.8 mL of distilled water under constant stirring. The suspension was incubated for 3 h at room temperature with stirring at 200 rpm and centrifuged. Then Hep_MIL-101 crystals were washed twice with double distilled water and dried under vacuum for 4 h.

Entrapment of Hep_MIL-101(Fe) into alumina sol-gel matrix.

1 mL of the alumina hydrosol (boehmite NPs mass fraction 2%) was mixed with 4 mg of the pre-purified Hep MIL-101 (Fe) powder with the mass ratio AlOOH:MIL-101(Fe) 5:1. The resulting mixture were dried in a vacuum desiccator at room temperature.

Creation of Hep_MIL-101(Fe)+SK@alumina hybrid.

1 mL of the alumina hydrosol (boehmite NPs mass fraction 2%) was mixed with 4 mg of the pre-purified Hep MIL-101 (Fe) powder with the mass ratio AlOOH:MIL-101(Fe) 5:1. To the resulting mixture 100 μL of streptokinase solution was added and mixed for 5 minutes. The resulting mixture were dried in a vacuum desiccator at room temperature.

Preparation of clots from human plasma.

Model clots were prepared from control human plasma with known amounts of fibrinogen and plasminogen. 10 μL of standard human plasma solution (plasminogen concentration was 102 μLg/mL and that for fibrinogen 2.8 mg/mL) was treated with 10 μL of thrombin solution (150 U/mL). The resulting mixture was gently stirred and allowed to stand for 5 minutes. The formed clot was separated and used in experiments.

Methods

Characterization methods.

X-ray powder diffraction (XRPD) characterization was performed on a D8 Advance Brucker diffractometer with Cu Kα1 radiation (λ = 1.54056 ˚A) and on a Siemens D5000 diffractometer with Cu *Kα1* radiation (λ = 1.54056 ˚A). Thermogravimetric analyses (TGA) of RT samples (510

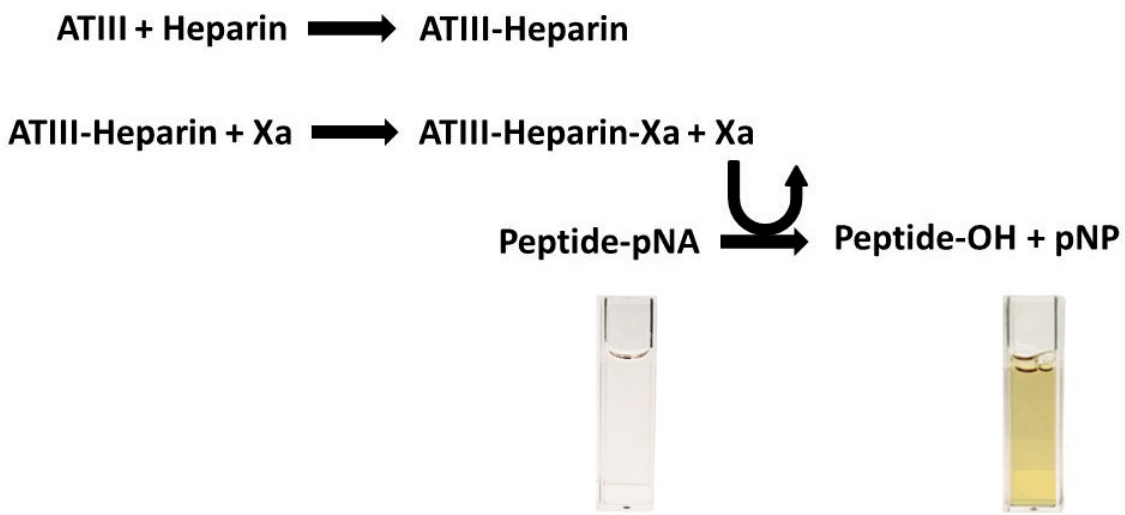
mg) were performed on a Perkin Elmer Diamond TGA/DTA STA 6000 under O2 atmosphere (20 mL min −1), at heating speed of 3◦C min −1 for the temperature range between room and 600◦C (note: heparin wt % is given with respect to dry MIL-100(Fe) weight). Particle size was monitored by Dynamic Light Scattering (DLS) on a Zetasizer Nano (Malvern Instruments). Samples were prepared by dispersing NPs (0.1 mg mL^{-1}, at room temperature) in the desired media using an ultrasound tip (10% amplitude for 1 min; Digital Sonifer 450, Branson). Transmission electron microscopy (TEM) was performed on a JEOL JEM-2100F at an accelerating voltage of 200 kV. Carbon-coated TEM grids, carbon type-B, 200 mesh copper grids were purchased from Ted Pella, Inc. Samples were prepared by dispersing dried NPs at 0.1 mg mL^{-1} in absolute ethanol using sonication, drop casting the solution on a carbon-coated TEM grid and allow the solvent to evaporate. SEM measurements were performed on a two-beam scanning microscope Tescan LYRA3 FEG. For measurements, the sample was deposited on the carbon tape, then carbon sputtering was carried out. The electronic image was obtained at low accelerating voltages (<20 kV) and low intensities of the probe, in order to diminish destruction of MOF crystals. Etching of the sample was carried out by gallium Focused Ion Beam (FIB) at the same sample without changing the sample angle. EDS measurements were performed by Oxford Instruments X-Max with a 150 mm2 sensor.

Spectroscopic determination of heparin release.

Direct heparin release determination was performed spectroscopically. For this purpose 6 mg of the Hep_MIL-101 (Fe) composite material (equivalent to 50 IU of heparin) was placed in a quartz cuvette containing 2 mL of phosphate buffered solution (PBS) pH 7.2, incubated at 37◦C and optical density was measured at 210 nm in a kinetic mode.

Detection of heparin release by anti-Xa assay.

The composite materials Hep_MIL-101 (Fe) or Hep_MIL-101 (Cr) in an amount equivalent to 50 IU heparin were placed in a cuvette containing 5 ml PBS supplemented with 250 mg of bovine serum albumin and stirred at 37◦C for 1 hour, then the nanocomposite particles were filtered off using Phenex 200 nm syringe filter. To the 100 μl supernatant, 33 μl of control plasma, 33 μl of antithrombin III (ATIII) solution and 133 μl of PBS solution were added. To the resulting mixture 200 μl of factor Xa solution was added followed by incubation for 5 min at 37◦C. After incubation 200 μl of chromogenic substrate solution was added and the mixture was incubated for an additional 5 min at 37◦C. Then 400 μl of 50% acetic acid was added and the optical density was measured at 405 nm. The mechanism of the anti-Xa assay is schematically illustrated in Scheme 1S.



Scheme 1S: Biochemical cascade lying realized in anti-Xa heparin assay.

Study of anticoagulant properties of Hep_MIL-101(Fe).

To measure the anticoagulant activity 1 mL of a standard human plasma was mixed with 12 mg of Hep MIL-101(Fe) biocomposite (equivalent to 100 IU of heparin) at at 37◦C, then 200 μl of thrombin solution was added. The clot formation was determined spectrophotometrically at a wavelength of 500 nm. Based on the data, a time course of thrombus formation was made. For control 1 mL of control plasma was incubated with 100 IU of heparin and threaded in a same manner.

Cytotoxicity of Hep MIL-101 biocomposites.

The HCT116 human colon adenocarcinoma cell line (American Type Culture Collection, USA) was propagated in Dulbecco modified Eagles medium supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 100 U/ mL penicillin and 100 μg/mL streptomycin (PanEco, Russia) at 37◦C, 5% CO2 in a humidified atmosphere. Concentrations of aqueous stock suspensions were 4 mg/mL (MIL-101(Cr)) and 3 mg/mL (MIL-101(Fe)) (stored at 4◦C). Suspensions were pipetted thoroughly, then serial dilutions in the medium were prepared immediately prior to addition to the cell culture. The cytotoxicity of new composites was determined as described.[2s] Briefly, cells (5x103 in 190 μl of medium) were plated into 96-well plates overnight. Each suspension (from the respective dilution) was added to wells. Ten final concentrations were tested: 0.08-40 μg/mL for MIL-101(Cr) and 0.06-30 μg/mL for MIL-101(Fe). Cells were incubated at 37◦C, 5% CO2 for 72 h followed by a colorimetric MTT test. Cell growth inhibition was calculated as the percentage of optical density in wells with the respective concentration of the new composite normalized to optical density of blanc (no composite) wells (100%). The antitumor drug doxorubicin (0.1-50 μM) was used as a positive control of cytotoxicity.

Computational details.

All calculations were carried out with Gaussian 09 D.01 program package. Geometry optimization was performed at the PBE/6-31G(d,p) level of theory. Bulk solvent effects were accounted for by using a PCM model with standard parameters for water. The PCM correction was applied during the geometry optimization. The DFT results were corrected for weak van der Waals interactions by using the semi-empirical D3 correction of Grimme. The computational results were further refined by single-point calcualations on the optimized structures carried out at the PBE-D3+PCM/6-311+G(d,p) level of theory. For calculating local interactions between the reactive anionic sites of heparin and potential exchange sites on the trimeric metal nodes of MIL-101, the

simplified models of the two components were used. The heparin polymer was modeled by a sodium form of the dominant monosaccharide GlcNS(65), while MIL-101 structure was approximated by a trinuclear metal core extracted from the single crystal structure of the MOF with the terephthalate units replaced by the benzoate anions (Figure 1b). To account for structural rigidity of the realistic crystalline material, constrains were imposed on the positions of hydrogen atoms at para positions of the benzoate ligands.

Density functional theory calculations.

All DFT calculations were carried out using the GGA-type PBE31 exchange-correlation functional as implemented in Gaussian 09 D.01 program.32 Previous benchmark studies evidenced the high accuracy of this method for the description of a wide range of chemical systems.[3s-5s] The all electron 6-31G(d) basis set was used for all atoms during the geometry optimization and vibrational analysis. The electronic energies were refined by single-point calculations with 6-311+G(d,p) basis set on all atoms. Van der Waals interactions were accounted for by using the dispersion-corrected DFT-D3 method proposed by Grimme.36 All complexes were treated as neutral species. Bulk solvent effects due to aqueous environment employed in the adsorption experiments were accounted for by using the implicit PCM solvent model[6s] with standard parameters for water solvent. The nature of the stationary points was evaluated from the analytically computed harmonic modes. No imaginary frequencies were found for the optimized structures, while all transition states exhibited a single imaginary frequency, corresponding to the eigenvector along the reaction path. The assignment of the transition state structure to a particular reaction path was tested by perturbing the structure along the reaction path eigenvector in the directions of the product and the reagent followed by geometry optimization. Given the very large size of both the MIL-101 unit cell structure and heparin polysaccharide and taking into account the focus of the current computational study on the local effects due to adsorption, cluster modeling approach was employed here. Heparin was approximated by a monosaccharide GlcN, 6SNR model in which the remaining four glycoside units were substituted by a methyl group. The negative charge of the sulphomoieties was compensated by Na+ cations. The MIL-101 structure was approximated by a Fe3O cluster with six benzoic acid residues cut from the periodic iron terephthalate MIL-101 structure. The terminating para-H atoms of the phenyl moieties were fixed to mimic structural rigidity of MOF during geometry optimization.

Anticoagulant properties of Hep_MIL-101(Fe)+SK@alumina hybrids.

In order to evaluate the anticoagulant properties of Hep MIL-101(Fe)+SK@alumina 1 mL of a standard human plasma was mixed with 50 mg of Hep MIL-101(Fe)+SK@alumina biocomposite (equivalent to 100 IU of heparin) at at 37◦C, then 200 μl of thrombin solution was added. The clot formation was determined spectrophotometrically at a wavelength of 500 nm. Based on the data, a time course of thrombus formation was made. For control 1 mL of control plasma was incubated with 100 IU of heparin and threaded in a same manner.

Thrombolytic properties of Hep_MIL-101(Fe)+SK@alumina hybrids.

The activity of the hybrid material was studied using an optical microscope. The clots were formed as described above with a subsequent deposition on the thrombolytic sol-gel coatings formed on a cover glass. Transmission mode was used to see any morphological changes in clots during analysis. The thrombolysis was monitored as a function of time. The thickness of clots was controlled with a 10 μm copper foil clamped between glasses. The pictures were taken every 5 minutes with a subsequent analysis.

Coagulation of plasma in circulation model.

PTFE vein implant (untreated, coated with SK@alumina or Hep MIL-101(Fe)+SK@alumina hybrids) with diameter of 4 mm was filled with 20 mL of standard human plasma and circled. Plasma were pumped with a peristaltic pump at 100 mL/min flow rate. To circulating plasma 0.5 mL of thrombin solution (equals 50 U) was added. After 1 hour of circulation the implant was cut and coagulation of plasma was evaluated.

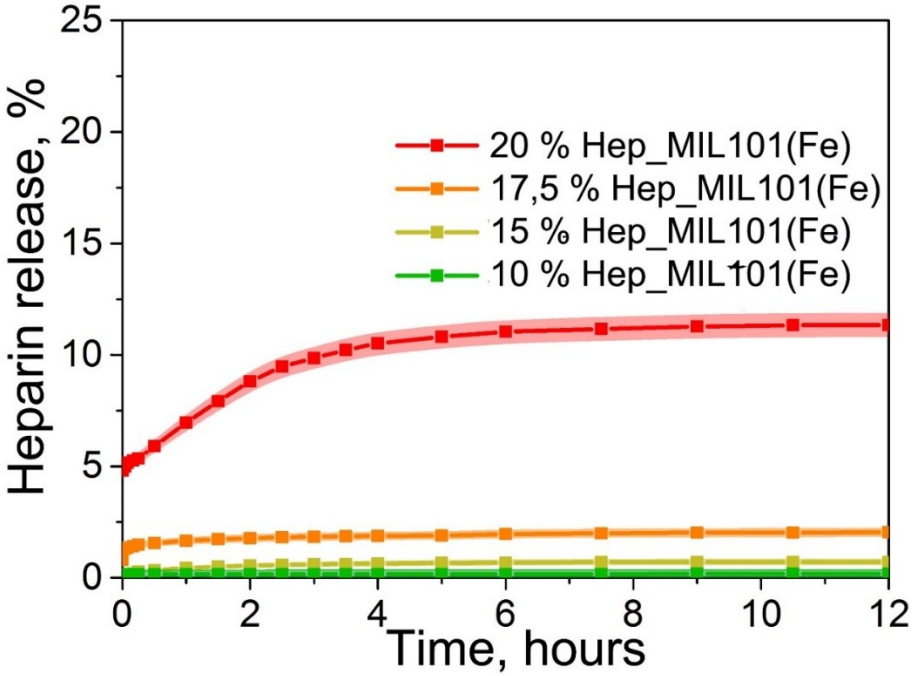


Figure 1S. Heparin release profile from Hep_MIL-101(Fe)

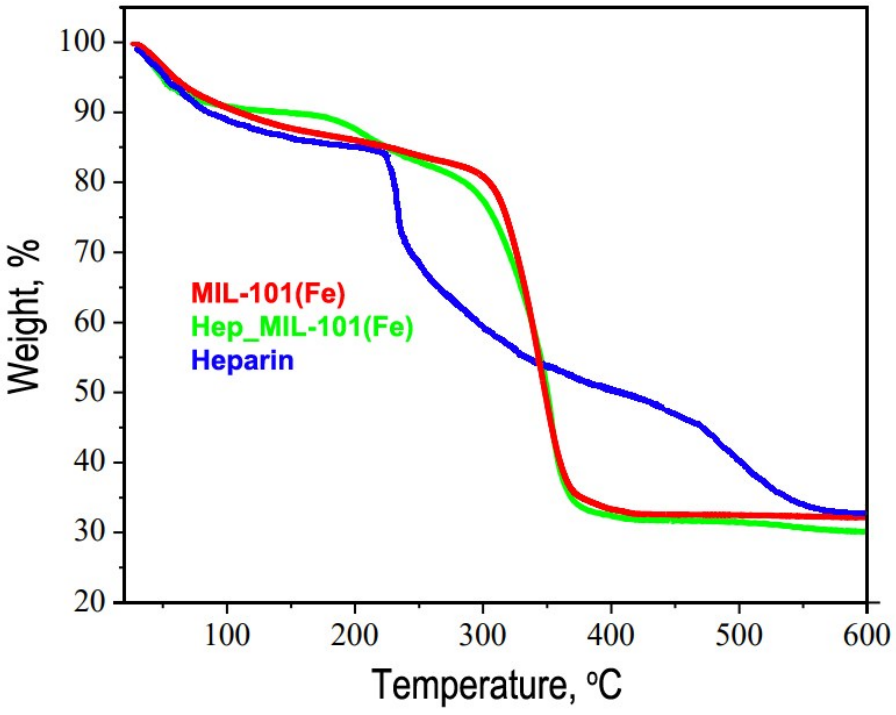


Figure 2S. Therogravimetric analysis of the materials

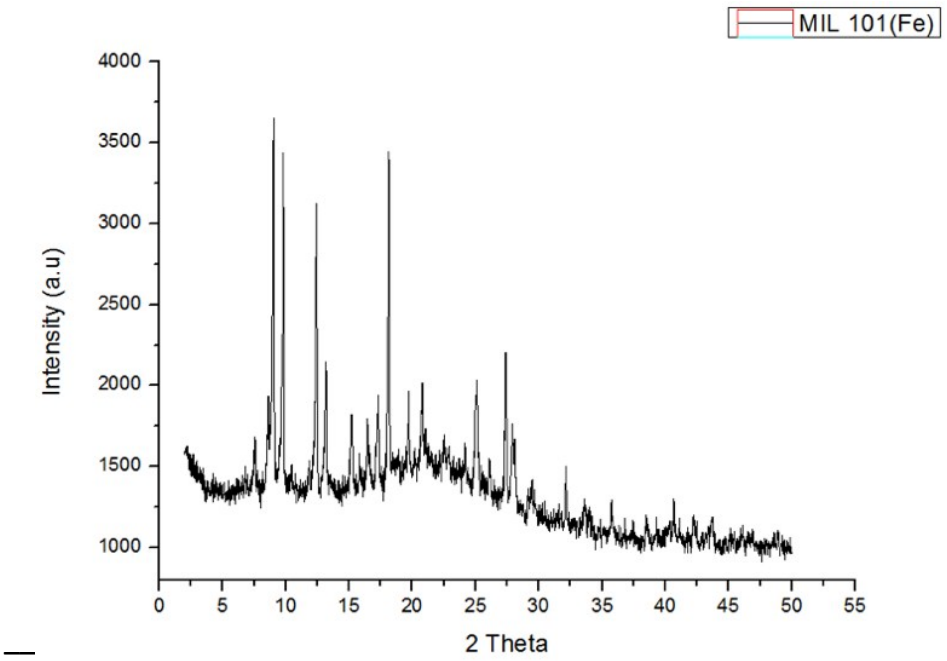


Figure 3S. XRD pattern of synthetized MIL-101(Fe)

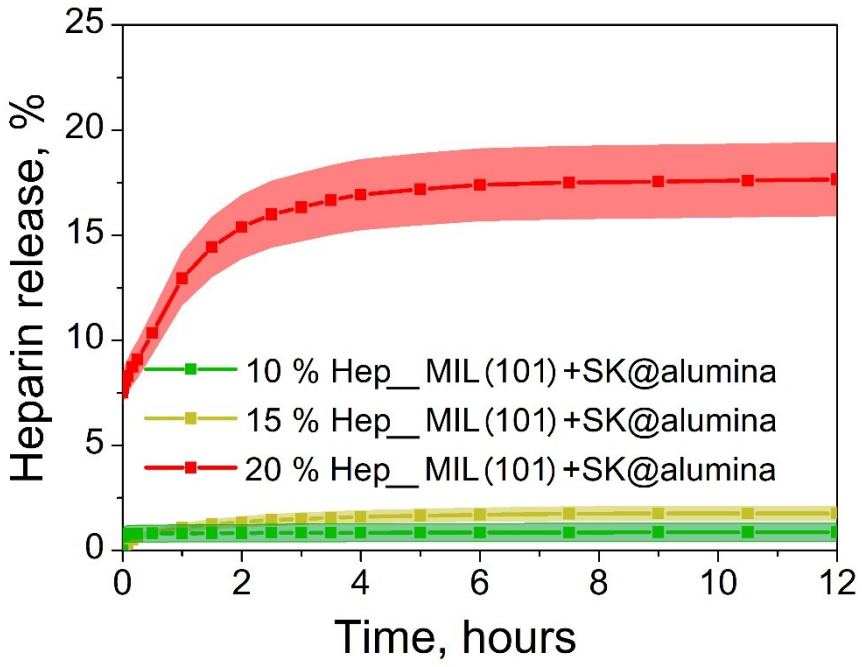


Figure 1S. Heparin release profile from Hep_MIL-101(Fe)

References

1S. Rutenberg, A.; Vinogradov, V. V.; Avnir, D. Synthesis and enhanced thermal stability of albumins@ alumina: towards injectable sol–gel materials. *Chemical Communications* **2013**, *49*, 5636–5638.

2S. Andreeva, Y. I.; Drozdov, A. S.; Fakhardo, A. F.; Cheplagin, N. A.; Shtil, A. A.; Vinogradov, V. V. The controllable destabilization route for synthesis of low cytotoxic magnetic nanospheres with photonic response. *Scientific Reports* **2017**, *7* .
3S. Perdew, J. P.; Burke, K.; Ernzerhof, M. Generalized gradient approximation made simple. *Physical review letters* **1996**, *77*, 3865.
4S. Frisch, M.; Trucks, G.; Schlegel, H. B.; Scuseria, G.; Robb, M.; Cheeseman, J.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. Gaussian 09, revision D. 01. 2009.
5S Zhao, Y.; Truhlar, D. G. Benchmark energetic data in a model system for Grubbs II metathesis catalysis and their use for the development, assessment, and validation of electronic structure methods. *Journal of chemical theory and computation* **2009**, *5*, 324–333.
6S. Valero, R.; Costa, R.; de PR Moreira, I.; Truhlar, D. G.; Illas, F. Performance of the M06 family of exchange-correlation functionals for predicting magnetic coupling in organic and inorganic molecules. *The Journal of chemical physics* **2008**, *128*, 114103.
7S Medvedev, M. G.; Bushmarinov, I. S.; Sun, J.; Perdew, J. P.; Lyssenko, K. A. Density functional theory is straying from the path toward the exact functional. *Science* **2017**, *355*, 49–52.
8S Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *The Journal of chemical physics* **2010**, *132*, 154104.
9S Tomasi, J.; Mennucci, B.; Cammi, R. Quantum mechanical continuum solvation models. *Chemical reviews* **2005**, *105*, 2999–3094.