Supporting Information

Binding of a Fatty Acid Functionalized Anderson-Type Polyoxometalate to Human Serum Albumin

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1. Experimental Section

All starting materials were purchased from Sigma-Aldrich, TCI, or Acros and used as received unless otherwise specified.

Fatty acid and globulin free HSA from Sigma-Aldrich (A3782) was used for the spectroscopic experiments (without further purification).

The educts $\text{Na}_3(\text{H}_2\text{O})_6[\text{Al(OH)}_3\text{Mo}_6\text{O}_{18}]\cdot2\text{H}_2\text{O}$ (AlMo$_6$) and $(\text{TBA})_3[\text{Al(OH)}_3\text{Mo}_6\text{O}_{18}]\{((\text{OCH})_2)_3\text{CNH}_2\}\cdot7\text{H}_2\text{O}$ (TBA-AlMo$_6$-Tris, TBA = tetrabutylammonium) were prepared according to reported procedures.$^{1,2}$

Powder X-ray diffraction was performed on an EMPYREAN diffractometer system using Cu Kα radiation ($\lambda= 1.540598$), a PIXcel3D-Medipix3 1x1 detector (used as a scanning line detector) and a divergence slit fixed at 0.1 mm. The scan range was from 5° to 50° (2θ).

$^1$H-NMR spectra were recorded on Bruker Avance III spectrometers at 500.32 and 500.10 MHz, respectively. $^1$H shifts are quoted relative to the solvent residual signals. Electrospray ionization (ESI) mass spectra were measured on a Bruker Esquire 3000 mass spectrometer. The measurement was carried out in methanol, whereby the data were collected in positive mode within the region of $m/z$ 100-1200. The $m/z$ values are quoted for the most abundant isotope.

Infrared (IR) spectra were obtained from a Bruker Vertex 70 FT-IR spectrometer by means of the attenuated-total-reflection (ATR) technique. All elemental analyses were carried out using an `EA 1108 CHNS-O' elemental analyzer (Carlo Erba Instruments) at the Microanalytical Laboratory of the University of Vienna.

Thermogravimetric analysis (TGA) was performed on a Mettler SDTA851e Thermogravimetric Analyzer under nitrogen flow, with a heating rate of 5 K/min in the region of 298-473 K. We performed a literature search using the SCOPUS (https://www.scopus.com/) and CCDC (https://www.ccdc.cam.ac.uk/structures/) databases to identify POMs functionalized by fatty acids, or more precisely, hybrid POMs containing aliphatic chains ($\geq$C3 atoms). The results of the literature search are summarized in Table S1.

1.1. Synthesis of Tris(hydroxymethyl)-lauroylamidomethan (Tris-lauroyl)

6 ml of triethyamine (43 mmol) was added to a cooled (12-14°C) solution of tris(hydroxymethyl)-aminomethane (Tris) (5.3 g, 44 mmol) in 250 ml methanol. Afterward, 46 mmol lauroyl chloride (10 g) was added dropwise over 20 minutes. The mixture was stirred overnight and the solvent was subsequently removed under vacuum. The residue was thoroughly washed with water, dried, washed with diethyl ether, once more with water and dried in vacuo. Yield: 6.1 g, 46%.

Anal. Calcd for C$_{16}$H$_{33}$NO$_4$ ($M_r = 303.44$ g/mol): C, 63.33; H, 10.96; N, 4.62; O, 21.09 %. Found: C, 63.59; H, 11.46; N, 4.63; O, 21.21 %.

IR (cm$^{-1}$): 3390s, 3281vs, 2917vs, 2850vs, 1623vs, 1535vs, 1468s, 1356s, 1286s, 1122s, 1062s, 1025vs, 955m, 862m, 720s.
1H-NMR (DMSO-d6, δ): 0.85 (t, 3H, CH3), 1.24 (s, 16 H, 8CH2), 1.46 (m, 2H, CH2), 2.12 (t, 2H, CH2), 3.51 (d, 6H, 3CH2), 4.77 (t, 3H, 3OH), 7.09 (s, 1H, NH).

ESI-MS in methanol (positive mode): 304 [M+H]+, 326 [M+Na]+.

1.2. Synthesis of (TBA)3[Al(OH)3Mo6O18(OCH2)3CNHCOC11H23]·9H2O (TBA-AlMo6-LA)

Route 1:

5 ml of Tris-lauroyl (0.38 g, 1.25 mmol) in dimethyl sulfoxide (DMSO) was added dropwise (over 1.5 hours) to a 40 ml solution of Na3(H2O)6[Al(OH)6Mo6O18]·2H2O (1.5 g, 1.24 mmol) in a DMSO-water mixture (3:1) at 100°C. The mixture was refluxed for further 4 hours before the solvents were removed at 80 °C under vacuum. The residue was washed with tetrahydrofuran (THF), redissolved in water, filtered, precipitated by adding TBA-Br solution, collected via vacuum filtration and dried on air. Yield: 1.3 g, 49 %.

IR (cm⁻¹): 3420br, 3338m, 2960s, 2928s, 2874s, 1667s, 1556s, 1481s, 1458s, 1380s, 1058s, 1028s, 940vs, 919vs, 900vs, 649vs, 567s, 445s, 368s, 325vs. The IR spectrum is shown in Figure S3.

1H-NMR (DMSO-d6, δ): 0.85 (t, 3H, CH3), 0.94 (t, 36H, 12CH3-TBA), 1.23 (s, 16 H, 8CH2), 1.32 (m, 24H, 12CH2-TBA), 1.40 (m, 2H, CH2), 1.57 (m, 24H, 12CH2-TBA), 1.97 (t, 2H, CH2), 3.17 (m, 24H, 12CH2-TBA), 3.50 (s, 3H, OH), 4.60 (d, 6H, 3CH2), 7.02 (s, 1H, NH). The 1H-NMR spectrum is shown in Figure S4.

Route 2:

0.26 ml of triethylamine (1.7 mmol) was added to a solution of (TBA)3[Al(OH)3Mo6O18(OCH2)3CNHCOC11H23]·7H2O (2.7 g, 1.5 mmol) in 20 ml of anhydrous acetonitrile. Afterward, 1.7 mmol lauroyl chloride (0.37 g) was added dropwise to that solution. The mixture was stirred at room temperature for 48 hours, filtered and evaporated under vacuum. The residue was washed with THF and water and recrystallized from a methanol-water mixture (1:1). Yield: 2.5 g, 77 %.

Anal. Calcd for C64H159AlMo6N4O34 (Mr = 2131.58 g/mol): C, 36.06; H, 7.52; N, 2.63 %. Found: C, 35.78; H, 7.68; N, 2.65 %.

TGA (see Figure S1) revealed that 7.71 % of the weight is lost when the compound is heated from 25 – 200 °C, which corresponds to nine water molecules.

IR and 1H-NMR spectra are identical to those of the product obtained by route 1.
1.3. Synthesis of Na₃[Al(OH)₃Mo₆O₁₈(OCH₂)₃CNHCOC₁₁H₂₃]+7H₂O (Na-AlMo₆-LA)

Na-AlMo₆-LA was obtained from TBA-AlMo₆-LA via ion-exchange using a column with Amberlite IR-120 in Na⁺-form and 50 % methanol as solvent.

Anal. Calcd for Na₃C₁₆H₄₇AlMo₆NO₃₂ (Mᵣ = 1437.12 g/mol): C, 13.37; H, 3.30; N, 0.97; O, 35.63 %. Found: C, 13.10; H, 3.48; N, 1.13; O, 34.52 %.

TGA revealed that 8.9 % of the weight is lost when the compound is heated from 25 to 200 °C, which corresponds to seven water molecules.

IR (cm⁻¹): 2923m, 2852m, 1631s, 1518s, 1460m, 1056s, 901vs, 633vs, 444s, 362s, 321s.

¹H-NMR (DMSO-d₆, δ): 0.85 (t, 3H, CH₃), 1.22 (s, 16 H, 8CH₂), 1.41 (m, 2H, CH₂), 1.98 (t, 2H, CH₂), 3.74 (s, 3H, 3OH), 4.60 (s, 6H, 3CH₂), 7.03 (s, 1H, NH).

1.4. Crystallization of HSA-Myr

Crystallization of HSA was only achieved in the presence of myristate (Myr) because the fatty acid-induced conformational changes facilitate the crystallization process of the protein. For this reason, HSA was dissolved in 50 mM potassium phosphate, pH 7.4, and 150 mM KCl, and incubated with a 10-fold excess of sodium myristate for 3 hours at 37 °C. Unbound myristate was removed by centrifugation at 4000 rpm for 15 min (at 4 °C). HSA-Myr was concentrated to 150 mg/ml in the above-mentioned buffer and subjected to crystallization. Initial crystallization trials were performed by the hanging-drop vapor-diffusion technique using a 15-well Easy-Xtal plate (Qiagen). Several microliters of the protein solution (100 – 200 mg/ml HSA-Myr) were mixed with 2 µl of reservoir solution (50 mM potassium phosphate, pH 7.5 – 8.0, 23-30 % PEG3350, 150 mM KCl) and incubated at 293 K. Initial crystals grew, however, in clusters and were highly twinned. Therefore, streak seeding was performed in drops that had been allowed to equilibrate for three days. Crystals continued to grow in clusters (even after three rounds of streak seeding) but were clearly less twinned. Thus, it was possible to detach single crystals for further crystallization experiments.

1.5. Crystallization of TBA-AlMo₆-LA

Initial crystals of TBA-AlMo₆-LA were obtained by redissolving the solid (solubility ~2 mM) in water and crystallization on air. However, the crystals were of relatively low quality, leading to incomplete structures during the subsequent structure solving process. High-quality crystals were obtained during the attempt to co-crystallize TBA-AlMo₆-LA with HSA-Myr. To crystallize the complex, the hanging-drop vapor-diffusion technique was applied using a 15-well Easy-Xtal plate (Qiagen). Single crystals of TBA-AlMo₆-LA grew at 293 K by mixing several microliters of protein solution (100 - 200 mg/ml HSA-Myr) with 2 µl of reservoir solution (50 mM potassium phosphate, pH 7.5 - 8.0, 23-30 % PEG3350, 150 mM KCl) and 2 µL of TBA-AlMo₆-LA (~2 mM). Crystals of TBA-AlMo₆-LA appeared within 24 hours. Interestingly, the presence of HSA was essential for the
formation of high-quality crystals of AlMo₆-LA because in the absence of the protein only small needles were obtained that were not suitable for X-ray diffraction.

1.6. X-ray diffraction experiment and structure elucidation of TBA-AlMo₆-LA

Crystals of TBA-AlMo₆-LA were mounted in nylon loops and flash-cooled in liquid nitrogen after a quick soak in a cryoprotectant solution (50 mM potassium phosphate, pH 7.5 - 8.0, 32 % PEG3350 and 150 mM KCl). X-ray data of TBA-AlMo₆-LA were measured on a Bruker D8 Venture diffractometer equipped with a multilayer monochromator, Cu K/α INCOATEC microfocus sealed tube, and an Oxford cooling system. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted at calculated positions and refined with the riding model; only the three hydrogen atoms at the POM core were refined free with the use of restraints. The following programs were used: Bruker SAINT software package³ using a narrow-frame algorithm for frame integration; SADABS⁴ for absorption correction; OLEX²⁵ for structure solution, refinement, and molecular diagrams; graphical user-interface Shelxl⁶ for refinement; graphical user-interface SHELXS-2015⁷ for structure solution; SHELXL-2015⁸ for refinement; Platon⁹ for symmetry check. Experimental data with the CCDC-Code (1944363) are available online: http://www.ccdc.cam.ac.uk/conts/retrieving.html. Crystal data, data collection parameters, and structure refinement details are given in Tables S2. Selected bond lengths are summarized in Table S3.

1.7. Fluorescence spectroscopy

Fluorescence emission spectra of HSA (5 µM) in absence and presence of various concentrations of TBA-AlMo₆-LA, AlMo₆, lauric acid, TBA, and Na-AlMo₆-LA (1.25, 2.5, 5, 10, 25, 50 and 125 µM) were measured on an Infinite 200 microplate reader (Tecan) using Microfluor 1Black flat-bottom microtiter plates (Thermo Scientific). Fluorescence measurements were performed at least in triplicates and at three different temperatures: 303, 308, and 310.5 K. The excitation and emission wavelengths were set at 270 nm and 320-450 nm, respectively. All samples were dissolved in 20 mM potassium phosphate (pH 7.4) and 150 mM KCl. Because TBA/Na-AlMo₆-LA and AlMo₆ exhibit substantial absorption at the excitation wavelength (and partially at the emission wavelengths) at high concentrations, the obtained fluorescence intensities had to be corrected for the inner filter effect. To account for the inner filter effect, the following formula was used:¹⁰

\[ F_{\text{corr}} = F_{\text{obs}} \times \text{antilog}[0.605 \times ((\text{OD}_{\text{ex}} + \text{OD}_{\text{em}})/2)] \]  

where \( F_{\text{corr}} \) and \( F_{\text{obs}} \) are the fluorescence intensities after and before correcting the inner filter effect, respectively, \( \text{OD}_{\text{ex}} \) and \( \text{OD}_{\text{em}} \) are the optical density at the excitation and emission wavelengths, respectively, and 0.605 is a factor considering the geometry of the plate wells.
The quenching effect of all quenchers (AlMo$_6$-LA, AlMo$_6$, and lauric acid) was evaluated by both the common and modified Stern-Volmer equations (see main text). The fluorescence spectra of HSA with AlMo$_6$-LA, AlMo$_6$, and lauric acid, respectively, are shown in the Figures S5-S8 and Figure 2 (main text).

1.8. CD spectroscopy

CD spectra were collected using a Chirascan Plus spectropolarimeter (Applied Photophysics). The apparatus was sufficiently purged with nitrogen gas before starting the experiment. All CD measurements were carried out at 303 K using a CS/PCS single cell controller. Each experiment was performed using a precision quartz cuvette of 0.1 cm pathlength, and data were collected at wavelengths between 200 and 260 nm. Every spectrum shown here is the average of five successive scans, whereby the data were baseline subtracted for buffer. All samples were prepared in 20 mM potassium phosphate buffer at pH 7.5 with 150 mM KCl and a constant HSA concentration of 0.25 mg/ml (3.76 µM). The concentration of TBA-AlMo$_6$-LA, AlMo$_6$, and lauric acid, respectively, varied from 3.76 to 14 µM. The α-helicity of free HSA and the HSA-AlMo$_6$-LA-, HSA-AlMo$_6$- and HSA-LA-complexes was calculated using the following equation $^{11}$:

$$\alpha\text{-helix} \, (\%) = [(\Theta_{222} - 3000)/(-36000 - 3000)] \times 100$$ (2)

where $\Theta_{222}$ is the observed mean residue ellipticity at 222 nm (in deg cm$^2$/dmol) and 36000 and 3000 are the Θ values of a pure α-helix, β-sheet and random coil conformation at 222 nm, respectively. Θ$_{222}$ was calculated from each spectrum using the following equation $^{12}$:

$$[\Theta] = \theta_{\text{obs}}/(10 \times n \times C_p \times l)$$ (3)

where $\theta_{\text{obs}}$ is the measured ellipticity in millidegrees (mdeg), $n$ is the number of amino acids in HSA, $C_p$ is the molar concentration of HSA and $l$ is the pathlength of light in cm of the cuvette. The α-helicity of each sample is summarized in Table S5.

1.9. Crystallization of HSA-Myr and subsequent soaking with TBA-AlMo$_6$-LA

HSA-Myr crystals were obtained as described in section 1.4. and transferred into stabilizing drops containing 50 mM potassium phosphate, pH 7.5 - 8.0, 35 % PEG3350, 150 mM KCl, and 2 mM Myr. Soaking of HSA-Myr crystals in TBA-AlMo$_6$-LA-containing drops did not yield crystals of the complex because of the low solubility of the hybrid POM (~ 2 mM). However, AlMo$_6$-LA was successfully introduced into the HSA structure by directly adding TBA-AlMo$_6$-LA powder into the stabilizing drop. After soaking overnight (longer soaking times had negative effects on the crystals), crystals were harvested in nylon loops, quickly wiped through a freshly prepared stabilizing drop to remove POM powder, and subsequently flash-frozen in liquid nitrogen. The complex was successfully formed in four of five trials; however, crystal soaking with unmodified AlMo$_6$ (using the same procedure) did not yield the complex structure (in four trials).
1.10. X-ray diffraction experiment and structure elucidation of HSA-Myr-AlMo₆-LA

X-ray data of the HSA-Myr-AlMo₆-LA complex were collected at 100 K using a BRUKER D8 VENTURE X-ray diffractometer equipped with a multilayer monochromator, a PHOTON II charge-integrating pixel array detector, and a Cu-Kα Incoatec Microfocus (sealed tube). Diffraction data were processed with XDS¹³, whereby two datasets of the same crystal were merged with XSCALE¹⁴ to obtain one dataset with reasonable completeness. Initial phases were obtained by the molecular replacement method applying PHASER¹⁵; PDB entry 1N5U¹⁶ was used as a search model. The obtained structure was refined with phenix.refine¹⁷ and occasionally manually edited with Coot¹⁸. After the refinement has reached convergence, two molecules of AlMo₆-LA (without TBA cations) were introduced into the structure with COOT. Coordinates and restraints for the POM were generated with phenix.elbow¹⁹ using the coordination file of TBA-AlMo₆-LA (CCDC entry 1944363) as input. The HSA-Myr-AlMo₆-LA structure was further refined until convergence, whereby the occupancies and the B-factors (using anisotropic ADPs for the metals) were separately refined for the POMs. Data collection and refinement statistics are summarized in Table S6.
2. Figures

![TGA curve](image)

**Figure S1.** TGA curve of TBA-AlMo₆-LA. The TGA curve shows a two-step weight loss process (difference plot is shown in red). The weight loss during the first step was due to dehydration (25-200 °C), where 7.71 % of the weight was lost, corresponding to nine lattice water molecules. During the second step, 1.03 % of the weight was lost, which might be due to AlMo₆-LA decomposition. Please note that the maximum temperature of 200 °C was reached after 45 minutes and was then kept constant until the end of the experiment.
Figure S2. X-ray powder diffractogram. The experimentally determined (blue, top) and simulated (black, bottom) X-ray diffraction patterns of TBA-AlMo$_6$-LA are shown.

Figure S3. IR spectrum of TBA-AlMo$_6$-LA.
Figure S4. $^1$H-NMR spectrum of TBA-AlMo$_6$-LA. The inset depicts the sample composition. A simplified structure of TBA-AlMo$_6$-LA is shown on the left side of the inset; however, the three protons attached to the three $\mu_3$-O atoms are indicated (a). On the right side of the inset, three TBA molecules are depicted with assigned protons.
Fluorescence quenching spectra of HSA in the presence of different concentrations of AlMo$_6$. Spectra were measured at A) 303 K, B) 308 K, and C) 310.5 K, respectively. The excitation wavelength was 270 nm, and the emission wavelength was in the range of 320-450 nm. c(HSA) = 5 µM, c(AlMo$_6$) = 1.25, 2.5, 5, 10, 25, 50 and 125 µM, respectively. Quenching was only observed at a temperature of 303 K and only at the highest tested concentration of AlMo$_6$ (125 µM).
Figure S6. Fluorescence quenching spectrum of HSA at different concentrations of A) lauric acid (LA) and B) TBA. Spectra were measured at 303 K. Excitation wavelength was 270 nm, and the emission wavelength was in the range of 320-450 nm. c(HSA) = 5 µM, c(FA and TBA) = 1.25, 2.5, 5, 10, 25, 50 and 125 µM, respectively. Lauric acid enhances the intrinsic fluorescence of HSA with increasing concentration. It is known that the binding of long-chain FAs (> C12) induces conformational changes in HSA, which results in the enhancement of the intrinsic fluorescence. On the other hand, TBA exhibited no significant effect on the protein’s fluorescence.

Figure S7. Fluorescence quenching of HSA by Na-AlMo₆-LA. A) Fluorescence quenching spectrum of HSA at different concentrations of Na-AlMo₆-LA. Spectrum was measured at 303 K. Excitation wavelength was 270 nm, and the emission wavelength was in the range of 320-450 nm. c(HSA) = 5 µM, c(Na-AlMo₆-LA) = 1.25, 2.5, 5, 10, 25, 50 and 125 µM, respectively. B) The Stern-Volmer plot of HSA fluorescence quenching by Na-AlMo₆-LA at 303 K. For comparison, the Stern-Volmer plot derived from the quenching experiment with TBA-AlMo₆-LA is also shown (grey plot). Both the fluorescence quenching spectrum and the Stern-Volmer plot indicate that the quenching of the sodium salt is very similar to that of the TBA salt. It can be concluded that the counterions have no significant effect on the quenching behavior of AlMo₆-LA.
Figure S8. Fluorescence quenching spectra of HSA at different concentrations of TBA-AlMo₆-LA. Spectra were measured at A) 308 K and B) 310.5 K, respectively. The excitation wavelength was 270 nm, and the emission wavelength was in the range of 320-450 nm. c(HSA) = 5 µM, c(TBA-AlMo₆-LA) = 1.25, 2.5, 5, 10, 25, 50 and 125 µM, respectively. The functionalized POM exhibited remarkable quenching because the intrinsic fluorescence of HSA was gradually decreased with increasing concentrations of TBA-AlMo₆-LA. In addition, a bathochromic shift of the emission maximum was observed, indicating a more polar environment of Trp214 (changes due to the binding of TBA-AlMo₆-LA).

Figure S9. Stern-Volmer plots of HSA fluorescence quenching by TBA-AlMo₆-LA at 303, 308, and 310.5 K. The slope of the plot decreases with increasing temperature, indicating static quenching.
Figure S10. Modified Stern-Volmer plots for HSA fluorescence quenching by TBA-AlMo₆-LA at A) 303 K, B) 308 K, and C) 310.5 K, respectively. The values for $K_A$ and $n$ can be obtained from the intercept and slope of each plot, respectively (see Table S4).
A) Ellipticity (mdeg)

Wavelength (nm)

B) Ellipticity (mdeg)

Wavelength (nm)

C) Ellipticity (mdeg)

Wavelength (nm)

**Figure S11.** CD spectra of the A) HSA-TBA-AlMo₆-LA-, B) HSA-LA-, and C) HSA-AlMo₆-complexes. The two negative bands in the ultraviolet region at 208 and 222 nm of the CD spectra are characteristic of the α-helical structure of HSA. The binding of TBA-AlMo₆-LA resulted in a significant decrease in the intensity of both of these bands, indicating a significant change in the α-helix content of the protein. The α-helix content decreased gradually from 65.1 % to 55.5 % with increasing concentrations of TBA-AlMo₆-LA. LA and AlMo₆ exhibited a distinctly lower effect on the secondary structure of HSA than TBA-AlMo₆-LA because the α-helix content was only reduced to 60.5 % and 61.7 %, respectively. These results show that TBA-AlMo₆-LA was bound to the main polypeptide chain of HAS because the hybrid POM has clearly perturbed the hydrogen bonding network of the protein.
Figure S12. Further crystallographic details about the crystal structure of the HSA-Myr-AlMo_6-LA. A) Electron density map (2mF_o-DF_c map contoured at 1.5σ) of the inorganic AlMo_6 core (Tris-lauroyl group is omitted for clarity). The disc-shaped electron density (shown as grey mesh) fits the POM molecule. B) The overall structure of the complex with the different domains of HSA being color-indicated. In addition, the location of Trp214 (shown as red sticks) and the fatty acid-binding sites (FA1-7) are indicated. TBA-AlMo_6-LA binds to the interdomain cleft of HSA and exhibits contacts with domain IIIA, which is one of the two major drug binding sites (Sudlow site II); domain IIB; and a large loop that connects domain IA with domain IB. The alkyl chain of AlMo_6-LA is sandwiched between helices of domain IB and IIIA, whereas the inorganic core interacts mainly with the before-described loop (including some hydrophobic interactions with one α-helix of domain IIIA). C) The interaction of the POM with HSA is described in more detail in the main text. C) The electrostatic surface potential of HSA. The electrostatic potential is measured in units of k_B T/e, with a range as shown in the color bar. The environment of the binding site of AlMo_6-LA (shown in ball-and-stick mode) is dominated by hydrophobic (white) and positively charged patches (blue) and therefore well suited to accommodate the POM with its negatively charged core and hydrophobic functionality. Please note that the second AlMo_6-LA molecule, which is bound at a crystal contact, was ignored in this discussion because it most probably represents only a crystallographic artifact.
### 3. Tables

**Table S1 – POMs containing alkyl chains (results of the literature search).**

| Compound                                                                 | POM-type | Ref. |
|--------------------------------------------------------------------------|----------|------|
| [MnMo$_6$O$_{18}${(OCH$_2$)$_3$C NH-C O-(CH$_2$)$_4$CH$_3$}]$_2$         | Anderson | 21,22|
| [MnMo$_6$O$_{18}${(OCH$_2$)$_3$C NH-C O-(CH$_2$)$_{14}$CH$_3$}]$_2$     | Anderson | 21,22|
| [MnMo$_6$O$_{18}${(OCH$_2$)$_3$C NH-C O-(CH$_2$)$_{16}$CH$_3$}]$_2$     | Anderson | 21,22|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C-(CH$_2$)$_7$CHCH$_2$}]$_2$           | Anderson | 23,24,22|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C-(CH$_2$)$_{14}$CH$_3$}((OCH$_2$)$_3$C NHCH$_2$C$_{16}$H$_6$)}]$_2$ | Anderson | 23,22|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C NH-C O-(CH$_2$)$_2$CHCH$_2$}]$_2$     | Anderson | 22|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C NH-C O-(CH$_2$)$_{10}$CH$_3$}]$_2$   | Anderson | 22|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C NH-C O-(CH$_2$)$_{12}$CH$_3$}]$_2$   | Anderson | 22|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C NH-C O-(CH$_2$)$_{18}$CH$_3$}]$_2$   | Anderson | 22|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C NH-C O-C$_6$H$_4$-O-C$_8$H$_{17}$}]$_2$ | Anderson | 25|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C NH-C O-C$_6$H$_2$-(O-C$_8$H$_{17}$)}]$_2$ | Anderson | 25|
| [(MnMo$_6$O$_{18}$){(OCH$_2$)$_3$C NH-C O-C$_6$H$_3$-(O-C$_8$H$_{17}$)}]$_2$ | Anderson | 25|
| [(Mo$_6$O$_{18}$)(NCH$_2$(CH$_2$)$_3$CH$_3$)]$_2$                         | Lindqvist| 26|
| [(Mo$_6$O$_{18}$)(NCH$_2$(CH$_2$)$_{16}$CH$_3$)]$_2$                    | Lindqvist| 26|
| [(V$_6$O$_{13}$){(OCH$_2$)$_3$C CO-O(CH$_2$)$_3$CH$_3$}]$_2$            | Lindqvist| 27|
| [(V$_6$O$_{13}$){(OCH$_2$)$_3$C CO-O(CH$_2$)$_{8}$CH=CH$_2$}]$_2$       | Lindqvist| 27|
| [(V$_6$O$_{13}$){(OCH$_2$)$_3$C NH-CO-(OCH$_2$)$_3$C NHCH$_2$C$_6$H$_4$-CO-O-C$_{16}$H$_{33}$}]$_2$ | Lindqvist| 28|
| [(PW$_{11}$O$_{39}$){Si(CH$_2$)$_n$CH$_3$}]$_2$ (n = 7, 11, 15, 17)      | Keggin   | 29|

Please note that all Anderson-type POMs in this table are double-side-functionalized structures (i.e., both sides of the POM core are functionalized).
| **Empirical formula** | \( \text{C}_{64}\text{H}_{138}\text{AlMo}_{6}\text{N}_{4}\text{O}_{35} \) |
| **CCDDC code**       | 1944363 |
| **Formula weight Mr (g/mol)** | 2127.40 |
| **Crystal system**    | \( P\overline{b}c\alpha \) |
| **Space group**       | Orthorhombic |
| **Temperature (K)**   | 100 |
| **a, b, c (Å)**       | 23.0350 (8), 23.1676 (7), 36.0086 (11) |
| **α, β, γ (°)**       | 90, 90, 90 |
| **V (Å\(^3\))**       | 19216.6 (11) |
| **Z**                 | 8 |
| **\( D_{\text{calc}} \) (g/cm\(^3\))** | 1.471 |
| **Radiation (wavelength)** | \( \text{CuK}\alpha (\lambda = 1.54178 \text{ Å}) \) |
| **Crystal size (mm\(^3\))** | 0.15 x 0.15 x 0.12 |
| **\( \mu \) (mm\(^{-1}\))** | 6.952 |
| **No. of reflections** | 198549 |
| **R\(_1\) (all data)** | 0.0945 |
| **wR (all data)**     | 0.2623 |
| **R\(_1\) (I>2σ(I))** | 0.0884 |
| **wR (I>2σ(I))**      | 0.2549 |
| **Goodness-of-fit on F\(^2\)** | 1.109 |
| **\( \rho_{\text{max}}, \rho_{\text{min}} \) (e Å\(^{-3}\))** | 1.59, -0.62 |
### Table S3 – Selected bond lengths in TBA-AlMo$_6$-LA.

| Bond          | Length (Å)          |
|---------------|---------------------|
| Mo-$\mu_3$-O  | 2.301 (5) – 2.365 (5) |
| Mo-$\mu_2$-O  | 1.918 (5) – 1.948 (5) |
| Mo-O$_t$      | 1.687 (6) – 1.720 (6) |
| Al-$\mu_3$-O  | 1.865 (5) – 1.925 (5) |
| $\mu_3$-O-C   | 1.425 (10) – 1.463 (9) |

### Table S4 – Binding constant and number of bound TBA-AlMo$_6$-LA molecules at various temperatures.

| Temp (K) | $K_A$ ($10^5$ M$^{-1}$)$^a$ | $n$$^b$ | $R^2$  |
|----------|-----------------------------|--------|--------|
| 303.0    | 1.56                        | 0.90   | 0.989  |
| 308.0    | 1.66                        | 0.83   | 0.986  |
| 310.5    | 1.06                        | 0.84   | 0.985  |

$^a$ $K_A$ = association constant

$^b$ $n$ = number of binding sites

The values of Table S4 were derived from Figure S10.
Table S5 – α-helicity of free HSA and the HSA-TBA-AlMo$_6$-LA-, HSA-AlMo$_6$, and HSA-LA-complexes, respectively.

| compound       | P : L ratio* | α-helix (%) |
|----------------|--------------|-------------|
| Free HSA       | -            | 65.06       |
| HSA-TBA-AlMo$_6$-LA | 1:1         | 62.54       |
|                | 1:5          | 61.16       |
|                | 1:10         | 58.52       |
|                | 1:25         | 55.52       |
| HSA-LA         | 1:1          | 65.62       |
|                | 1:5          | 64.64       |
|                | 1:10         | 62.70       |
|                | 1:25         | 60.45       |
| HSA-AlMo$_6$   | 1:1          | 63.96       |
|                | 1:5          | 63.83       |
|                | 1:10         | 62.47       |
|                | 1:25         | 61.65       |

*Protein : Ligand ratio with L = AlMo$_6$-LA, AlMo$_6$ or LA, respectively.
Table S6 – Crystallographic data for the HSA-Myr-AlMo<sub>6</sub>-LA complex.

| Property                                      | Value                    |
|----------------------------------------------|--------------------------|
| Space group                                  | C 1 2 1                  |
| a, b, c (Å)                                  | 184.65, 38.54, 96.06     |
| α, β, γ (°)                                  | 90.0, 105.1, 90.0        |

**Data collection and processing**

| Property                                      | Value                    |
|----------------------------------------------|--------------------------|
| Wavelength (Å)                               | 1.54178                  |
| Resolution range (Å)                         | 31.89 – 3.00 (3.107 – 3.00) |
| No. of total reflections                     | 56248 (4719)             |
| No. of unique reflections                    | 13029 (1252)             |
| Multiplicity                                 | 4.3 (3.8)                |
| Completeness (%)                             | 96.13                    |
| Mean I/σ                                     | 14.37 (3.76)             |
| R<sub>p.i.m.</sub> [a]                        | 0.045 (0.175)            |
| R<sub>merge</sub> [b]                         | 0.085 (0.312)            |
| CC<sub>1/2</sub> [c]                          | 0.995 (0.905)            |

**Refinement statistics**

| Property                                      | Value                    |
|----------------------------------------------|--------------------------|
| No. of reflections used in refinement        | 13029 (1252)             |
| R<sub>work</sub> [d]                          | 0.2692 (0.3299)          |
| R<sub>free</sub> [e]                          | 0.3112 (0.3322)          |
| Average B-factor                             | 48.42                    |
| Macromolecule B-factor                       | 49.49                    |
| Ligands B-factor                             | 21.10                    |

**Ramachandran plot**

| Property                                      | Value                    |
|----------------------------------------------|--------------------------|
| Ramachandran favored (%)                     | 97.24                    |
| Ramachandran allowed (%)                     | 2.76                     |
| Ramachandran outliers (%)                    | 0.00                     |
| PDB entry                                    | 6XV0                     |

---

[a] R<sub>p.i.m.</sub> = Σ<sub>hkl</sub> [n/(n–1)]<sup>1/2</sup> · Σ<sub>i</sub> |I<sub>i</sub>(hkl) - <I(hkl)>| / Σ<sub>hkl</sub> Σ<sub>i</sub> |I<sub>i</sub>(hkl)|, where n is the multiplicity, I<sub>i</sub>(hkl) is the ith observation of reflection hkl and is the weighted average intensity for all observations of reflection hkl.

[b] R<sub>merge</sub> = Σ<sub>hkl</sub> |I<sub>1</sub>(hkl) - <I(hkl)>| / Σ<sub>hkl</sub> |I<sub>1</sub>(hkl)|

[c] The mean intensity correlation coefficient of half-data sets. Values in parentheses are for the highest resolution shell.

[d] R<sub>work</sub> = Σ [F<sub>calc</sub> - |F<sub>obs</sub>| / |F<sub>obs</sub>| · 100, where F<sub>calc</sub> and F<sub>obs</sub> are the calculated and observed factor amplitudes, respectively.

[e] R<sub>free</sub> is calculated using a randomly chosen reference set of 5% of all the reflections collected.
4. References

(1) Shivaiah, V.; Das, S. K. Supramolecular Assembly Based on a Heteropolyanion: Synthesis and Crystal Structure of Na$_3$(H$_2$O)$_6$[Al(OH)$_6$Mo$_6$O$_{18}$]-2H$_2$O. *J. Chem. Sci.* **2005**, *117* (3), 227–233.

(2) Ai, H.; Wang, Y.; Li, B.; Wu, L. Synthesis and Characterization of Single-Side Organically Grafted Anderson-Type Polyoxometalates. *Eur. J. Inorg. Chem.* **2014**, *2014* (17), 2766–2772.

(3) Bruker SAINT V8.32B Copyright © 2005-2015 Bruker AXS.

(4) Krause, L.; Herbst-Irmer, R.; Sheldrick, G. M.; Stalke D. Comparison of silver and molybdenum microfocus X-ray sources for single-crystal structure determination. *J. Appl. Cryst.* **2015**, 48, 3–10.

(5) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. a. K.; Puschmann, H. OLEX2: A Complete Structure Solution, Refinement and Analysis Program. *J. Appl. Cryst.* **2009**, *42* (2), 339–341.

(6) Hübschle, C. B.; Sheldrick, G. M.; Dittrich, B. *SHELXle*: A Qt Graphical User Interface for *SHELXL*. *J. Appl. Cryst.* **2011**, *44* (6), 1281–1284.

(7) Sheldrick, G. M. SHELXS-2015 http://shelx.uni-ac.gwdg.de/SHELX/index.php.

(8) Sheldrick, G. M.; Crystal structure refinement with *SHELXL*. *Acta Cryst. C* **2015**, *71*, 3–8.

(9) Spek, A. L. Structure Validation in Chemical Crystallography. *Acta Cryst. D* **2009**, *65* (2), 148–155.

(10) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer US, 2006.

(11) Yang, P.; Gao, F. Principle of Bioinorganic Chemical, Science Press, **2002**.

(12) Johnson, W. C. Protein Secondary Structure and Circular Dichroism: A Practical Guide. *Proteins: Structure, Function, and Genetics* **1990**, *7* (3), 205–214.

(13) Kabsch, W. XDS. *Acta Crystallogr D Biol Crystallogr* **2010**, *66* (Pt 2), 125–132.

(14) Kabsch, W. Integration, Scaling, Space-Group Assignment and Post-Refinement. *Acta Cryst. D Biol. Crystallogr.* **2010**, *66* (Pt 2), 133–144.

(15) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser Crystallographic Software. *J. Appl. Cryst.* **2007**, *40* (4), 658–674.

(16) Wardell, M.; Wang, Z.; Ho, J. X.; Robert, J.; Rucker, F.; Ruble, J.; Carter, D. C. The Atomic Structure of Human Methemalbumin at 1.9 Å. *Biochemical and Biophysical Research Communications* **2002**, *291* (4), 813–819.

(17) Afonine, P. V.; Grosse-Kunstleve, R. W.; Echols, N.; Headd, J. J.; Moriarty, N. W.; Mustyakov, M.; Terwilliger, T. C.; Urzhumtsev, A.; Zwart, P. H.; Adams, P. D. Towards Automated Crystallographic Structure Refinement with Phenix.Refine. *Acta Cryst. D* **2012**, *68* (4), 352–367.

(18) Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. Features and Development of Coot. *Acta Cryst. D* **2010**, *66* (4), 486–501.
(19) Moriarty, N. W.; Grosse-Kunstleve, R. W.; Adams, P. D. Electronic Ligand Builder and Optimization Workbench (ELBOW): A Tool for Ligand Coordinate and Restraint Generation. Acta Cryst. D 2009, 65 (10), 1074–1080.

(20) Curry, S.; Mandelkow, H.; Brick, P.; Franks, N. Crystal Structure of Human Serum Albumin Complexed with Fatty Acid Reveals an Asymmetric Distribution of Binding Sites. Nature Structural Biology 1998, 5 (9), 827–835.

(21) Song, Y.-F.; McMillan, N.; Long, D.-L.; Thiel, J.; Ding, Y.; Chen, H.; Gadegaard, N.; Cronin, L. Design of Hydrophobic Polyoxometalate Hybrid Assemblies Beyond Surfactant Encapsulation. Chem. – Eur. J. 2008, 14 (8), 2349–2354.

(22) Rosnes, M. H.; Musumeci, C.; Yvon, C.; Macdonell, A.; Pradeep, C. P.; Sartorio, C.; Long, D.-L.; Pignataro, B.; Cronin, L. Exploring the Interplay Between Ligand Derivatisation and Cation Type in the Assembly of Hybrid Polyoxometalate Mn-Andersons. Small 2013, 9 (13), 2316–2324.

(23) Rosnes, M. H.; Musumeci, C.; Pradeep, C. P.; Mathieson, J. S.; Long, D.-L.; Song, Y.-F.; Pignataro, B.; Cogdell, R.; Cronin, L. Assembly of Modular Asymmetric Organic–Inorganic Polyoxometalate Hybrids into Anisotropic Nanostructures. J. Am. Chem. Soc. 2010, 132 (44), 15490–15492.

(24) Musumeci, C.; Rosnes, M. H.; Giannazzo, F.; Symes, M. D.; Cronin, L.; Pignataro, B. Smart High-κ Nanodielectrics Using Solid Supported Polyoxometalate-Rich Nanostructures. ACS Nano 2011, 5 (12), 9992–9999.

(25) Lin, C.-G.; Chen, W.; Song, Y.-F. Self-Organization of Anderson-Based Amphiphiles. Eur. J. Inorg. Chem. 2014, 21, 3401–3405.

(26) Li, Q.; Wang, L.; Yin, P.; Wei, Y.; Hao, J.; Zhu, Y.; Zhu, L.; Yuan, G. Convenient Syntheses and Structural Characterizations of Mono-Substituted Alkylimido Hexamolybdates: [Mo$_{6}$O$_{18}$(NR)$_{2}$] (R = Me, Et, n-Pr, i-Pr, n-Bu, t-Bu, Cy, Hex, Ode). Dalton Trans. 2009, 7, 1172–1179.

(27) Bayaguud, A.; Li, J.; She, S.; Wei, Y. A Simple Synthetic Route to Polyoxovanadate-Based Organic–Inorganic Hybrids Using EEDQ as an Ester Coupling Agent. Dalton Trans. 2017, 46 (14), 4602–4608.

(28) Yin, P.; Bayaguud, A.; Cheng, P.; Haso, F.; Hu, L.; Wang, J.; Vezenov, D.; Winans, R. E.; Hao, J.; Li, T.; et al. Spontaneous Stepwise Self-Assembly of a Polyoxometalate–Organic Hybrid into Catalytically Active One-Dimensional Anisotropic Structures. Chem. – Eur. J. 2014, 20 (31), 9589–9595.

(29) Landsmann, S.; Lizandara-Pueyo, C.; Polarz, S. A New Class of Surfactants with Multinuclear, Inorganic Head Groups. J. Am. Chem. Soc. 2010, 132 (14), 5315–5321.