Ten Good Reasons for the Use of the Tellurium-Centered Anderson–Evans Polyoxotungstate in Protein Crystallography

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CONSPECTUS: Protein crystallography represents at present the most productive and most widely used method to obtain structural information on target proteins and protein–ligand complexes within the atomic resolution range. The knowledge obtained in this way is essential for understanding the biology, chemistry, and medicinal interest. Here, we address the very central problem in protein crystallography: the unpredictability of the crystallization process. Obtaining protein crystals that diffract to high resolutions represents the essential step to perform any structural study by X-ray crystallography; however, this method still depends basically on trial and error making it a very time- and resource-consuming process. The use of additives is an established process to enable or improve the crystallization of proteins in order to obtain high quality crystals. Therefore, a more universal additive addressing a wider range of proteins is desirable as it would represent a huge advance in protein crystallography and at the same time drastically impact multiple research fields. This in turn could add an overall benefit for the entire society as it profits from the faster development of novel or improved drugs and from a deeper understanding of biological, biochemical, and pharmacological phenomena.

With this aim in view, we have tested several compounds belonging to the emerging class of polyoxometalates (POMs) for their suitability as crystallization additives and revealed that the tellurium-centered Anderson–Evans polyoxotungstate [TeW₆O₂₄]⁶⁻ (TEW) was the most suitable POM-archetype. After its first successful application as a crystallization additive, we repeatedly reported on TEW’s positive effects on the crystallization behavior of proteins with a particular focus on the protein–TEW interactions. As electrostatic interactions are the main force for TEW binding to proteins, TEW with its highly negative charge addresses in principle all proteins possessing positively charged patches. Furthermore, due to its high structural and chemical diversity, TEW exhibits major advantages over some commonly used crystallization additives. Therefore, we summarized all features of TEW, which are beneficial for protein crystallization, and present ten good reasons to promote the use of TEW in protein crystallography as a powerful additive. Our results demonstrate that TEW is a compound that is, in many respects, predestined as a crystallization additive. We assume that many crystallographers and especially researchers, who are not experts in this field but willing to crystallize their structurally unknown target protein, could benefit from the use of TEW as it is able to promote both the crystallization process itself and the subsequent structure elucidation by providing valuable anomalous signals, which are helpful for the phasing step.

1. THE USAGE OF THE ANDERSON–EVANS POLYOXOTUNGSTATE AS AN ADDITIVE TO GROW PROTEIN CRYSTALS FOR X-RAY STRUCTURE DETERMINATION

1.1. X-ray Crystallography—A Powerful Method To Gain Important Structural Information

Biological macromolecules are essential for the myriad of biological functions of all living organisms. As the properties and functions of macromolecules can be derived from their 3D structure, macromolecular structure determination has gained immense importance, especially for research fields working on pharmaceutical and medicinal issues. The design and mode of action of most of the pharmaceutically active compounds depend on structural knowledge revealing relevant drug–protein interactions. This information, gained from single crystal X-ray diffraction, adds an overall benefit to the entire society as it profits from the faster development of improved drugs. According to the Protein Data Bank (PDB, www.rcsb.org) X-ray crystallography is by far the most applied method for macromolecular structure elucidation and responsible for about 90% of all PDB entries. Despite this high deposition number, crystallography is still a trial and error based method¹ and represents mainly a quite time-, cost-, and material-consuming procedure requiring typically milligram amounts of highly pure and homogeneous protein preparations. The most limiting factor is the obtaining of single crystals of sufficiently high
quality as the crystallization process is affected by a large number of physical parameters (e.g., component concentrations, pH, temperature, ionic strength, humidity, etc.), which are partially hardly controllable, leading to the unpredictability of the crystallization outcome.

1.2. The Use of Additives To Grow Protein Crystals

One of the easiest attempts to improve the crystallization probability of a macromolecule is the application of so-called additives. Additives are small compounds or molecules that are able to interact with the protein in a crystal assembly promoting manner and thus can exhibit dramatic influence on the crystallization process. On a purely rational basis, the best additives are those that are physiologically relevant for the protein like coenzymes, substrates, inhibitors, etc. as they are able to induce more stable or favorable conformations that are in turn mostly more likely to crystallize than the ligand-free form of the protein. These additives are, however, proteinspecific and thus merely restrictedly applicable. Other additives like charged groups or molecules or ions are able to promote crystallization by providing intermolecular, noncovalent crosslinks of electrostatic nature between protein molecules but it is mostly impossible to predict which compound under which conditions will lead to such beneficial interactions. Therefore, an universal additive with a rich repertoire of crystal packing affecting properties and addressing a larger group of macromolecules would be a groundbreaking advance in protein crystallography including all research disciplines relying on structural input.

1.3. A “Simple” Inorganic Cluster You Should Try as Crystallization Additive

During the search for a potential candidate for such a universal additive, our group examined a series of polyoxometalates (POMs) with regard to their ability to enhance the crystallization rate of some proteins. POMs are polynuclear metaloxide anions with an unparalleled diversity in structure and chemistry resulting in applications in many different research areas. During our investigation, one POM archetype particularly excelled in its ability to act as crystallization additive, namely, the Anderson–Evans type polyoxotungstate (POT) [TeW6O24]6− (TEW). TEW led to the crystallization of two hitherto structurally unknown proteins, mushroom tyrosinase from Agaricus bisporus (abPPPO4)8−, and aureone synthase from Coreopsis grandiflora (cgAUS1), and the model protein hen-egg white lysozyme (HEWL) into a previously unknown crystal form. TEW was found to mediate and stabilize crystal contacts by electrostatically (including H-bonds) cross-linking protein monomers and was therefore able to facilitate crystal lattice formation. These and other properties of TEW, which are the main part of this Account, contributed greatly to protein crystallization and the subsequent structure elucidation process. Based on our success with TEW, we think that the usage of this compound as crystallization additive is highly justified presenting the only existing (but highly important) application of the pure inorganic Anderson–Evans POT on a molecular level. Therefore, this Account aims to highlight the crystallization promoting features of TEW in order to approach protein crystallographers or scientists in general who are willing to elucidate the structure of their target protein, and we address the ever increasing POM community since we describe a POM-based application. Finally, we will give an outlook about possible extension of its usage by modifying the inorganic core of TEW representing an additional favorable feature of this POM archetype.

2. THE ANDERSON–EVANS POLYOXOTUNGSTATE ARCHETYPE

2.1. Inorganic Anderson-type Structure

The Anderson–Evans (AE) cluster is one of the pioneering POM archetypes and its structure was anticipated by J. S. Anderson in 1937; however, it was not until 1948 that the structure was crystallographically confirmed and later in 1974 finalized by H. T. Evans. The Anderson–Evans polyoxoanion (Figure 1) is composed of six edge-sharing Mo6 octahedra (M = addenda atoms, Mo or W) enclosing an octahedrally arranged heteroatom XO6 (X = most commonly transition metals) via edge-sharing leading to a planar structure that exhibits an approximate D3d symmetry. Six of the altogether 24 oxygen atoms are triple-bridged (µ3-O) connecting the heteroatom and two addenda atoms, another six oxygen atoms are double-bridged (µ2-O) connecting two addenda atoms, and the remaining 12 oxygen atoms are terminal oxygens (Oδ−), which are pairwise bound to each of the six addenda atoms. There exist two types of the Anderson–Evans structure, namely, the nonprotonated A-type with the heteroatom exhibiting its highest oxidation state ([(XII)Mo6O24]12− (e.g., X = TeVI, FFeIII)) and the protonated B-type, where the heteroatom is found in a lower oxidation state and the structure contains up to six protons on the µ3-O atoms ([(XIII)OH2]Mo6O18]6− (e.g., X = CrIII, FeIII)).

The focus of our research group lies on TEW ([TeW6O24]6−), which was successfully applied as a crystallization additive. The POT fulfills the most important prerequisites of a crystallization additive: (i) high solubility, (ii) stability under most crystallization conditions, (iii) the ability to interact with the protein, and (iv) maintenance of the protein’s integrity. The application of the pure inorganic POT in protein crystallography is so far the only successful application field as this polyoxoanion is extensively employed as an inorganic building block for the synthesis of hybrid organic–inorganic POMs.

2.2. Hybrid Organic–Inorganic Anderson-type Structures

In 2002, organically functionalized Anderson-type polyoxometalates (POMoxes) gained attention when the first Anderson-type hybrid structure was reported by Hasenkopf. This functionalization is achieved by replacing three or six protons of the B-type Anderson–Evans structure with organic tris-ligands (tris = tris(hydroxymethyl)methane, (RC(CH2OH)3)3). Since then this research field has rapidly grown and single- or double-
side grafted δ-, χ-, or ψ-isomers of functionalized Anderson-type POMs, [M(OH)₆Mo₆O₁₈]ₙ⁻ (M = Cu²⁺, Zn²⁺, Ni²⁺, Cr³⁺, Mn³⁺, Al³⁺, Fe³⁺, Ga³⁺), can be obtained via the rearrangement of octamolybdate or by applying a presynthesized Anderson–Evans polyanion under different reaction conditions containing the organic ligand. Recently, the first tris-functionalized POT has been described allowing now the synthesis of tailor-made hybrid POMs containing organic functionalities that could lead to specific interactions with the protein, for example, hydrophobic interactions (see section 4.2).

3. TEN GOOD REASONS TO USE THE ANDERSON–EVANS POLYOXOTUNGSTATE (TEW) IN PROTEIN CRYSTALLOGRAPHY

3.1. The Use of Tungsten Atoms to Solve the Phase Problem

In recent decades, POTs have been used in protein crystallography to overcome the phase problem. The introduction of heavy atoms or anomalous scatterers is the method of choice for the structure elucidation of proteins lacking a homologue structure. After the introduction of heavy or anomalously scattering atoms into the protein structure (e.g., by soaking), initial phases can be obtained by single or multiple isomorphous replacement applying heavy atoms (SIR, MR) or by single- or multiple-wavelength anomalous dispersion using anomalous scatterers (SAD, MAD). In all these methods, the phases are calculated based on differences in the crystals’ scattering behavior, which are introduced by either the heavy atoms (SIR, MR) or anomalous scatterers (SAD, MAD). POTs are excellent phasing tools as they represent clusters of numerous anomalously scattering heavy atoms that provide signals that significantly differ from those of the native data set enabling phase determination. Even at poor resolutions, where the single metal atom positions of metal clusters cannot be resolved or the weak signal gets lost in the noise, POT can act as a “superatom” delivering still useful phases, which is an advantage over commonly used single heavy atoms like Hg²⁺, Au³⁺, or Pt⁴⁺/⁴⁺. In the past, a series of polyoxotungstate-phosphates archetypes like the Wells–Dawson POT (K₆[P₂W₁₈O₆₂]), Keggin POT ((H₂O)₅[P₂W₁₈O₆₂]), and Preyssler POT (H₁₄[Na₅P₇W₃₃O₁₁₀]) were used to obtain heavy atom derivatives. We successfully applied TEW as a phasing tool during the structure elucidation of mushroom tyrosinase abPPO₄ and HEWL. A combination of molecular replacement (MR), a method deducing initial phases from the structure of a homologous protein, and SAD (MR-SAD) was applied during each structure determination. The use of the MR method alone would have been sufficient to solve those structures since good phases were derived from the respective homologue structure; however, exploiting the significant anomalous signal of TEW has improved the phase in each case and reduced model bias to a minimum, which is a common problem in MR as structural features of the homologue structure can contaminate and overlap the map of the structure of interest.

3.2. The High Solubility of TEW in Aqueous Solutions

One of the most important prerequisites of crystallization additives is, of course, a high solubility in aqueous solution as most additives are used in excess of the protein. The solubility of the sodium salt of TEW is approximately 100 mM and thus a wide range of TEW concentrations can be applied. Other POM archetypes like the Keggin and Wells–Dawson compounds are in general less soluble and thus less suitable for crystallization as they exhibit a solubility mainly in the range of 2–10 mM according to our experience. However, as the literature lacks exact description of the solubility for most POMs, the existence of POMs, exhibiting similar or even better solubility than TEW cannot be excluded (e.g., some Lindqvist-type niobates can easily reach a solubility of 20 mM). In general, the water solubility of POTs can be tuned by altering the counterion (e.g., H¹, Na¹, K¹, or Li¹).

3.3. The pH Stability of TEW

According to the PDB, proteins have been crystallized within the pH range of 2–10, whereby most of them were crystallized at pH 4–9. Thus, it is desirable that the additive largely covers this pH range in order to be widely applicable. In our experiments, TEW was stable over a period of several weeks to months at slightly alkaline and acidic pH values preserving its intact form as confirmed by crystal structures showing no hint for the formation of other protein-interacting tungsten species. The stability was tested at the common crystallization temperatures of 4 and 20 °C, respectively, and at TEW concentrations ranging from 1 to 20 mM indicating that the stability is fairly independent of the used concentration. In particular, TEW proved to be stable at pH = 7.5 (used for the crystallization of abPPO₄) at pH = 5.0 (used for the crystallization of cgAUS1) and at pH = 4.8 (used for the crystallization of HEWL). Therefore, it can be surely recommended to use TEW from pH 4.5 to 7.5 in aqueous solution covering a relative wide pH range.

3.4. TEW Preserves the Integrity of the Protein

Crystallization additives should not harm the protein in any way that could lead to its precipitation or denaturation during the crystallization trial. X-ray structure determination and SDS-PAGE experiments of TEW-protein complexes proved so far no conformational or significant chemical changes of the respective proteins. This should always be tested when considering the introduction of POMs into the crystallization mother liquor as some POMs tend to hydrolytically cleave proteins like lacunary POTs containing strong Lewis acids, which were shown to regioselectively cleave proteins and were therefore classified as artificial proteases. The nonhydrolytic activity of TEW is given by the circumstance that the central heteroatom, tellurium, is incorporated in the planar disk-shaped Anderson framework (Figure 1) and is thus not able to interact directly with the protein. This shielding of the central heteroatom is achieved by crystal structures showing no hint for alteration of the respective proteins. Nevertheless, it is desirable that the additive largely covers this pH range in order to be widely applicable. In our experiments, TEW was stable over a period of several weeks to months at slightly alkaline and acidic pH values preserving its intact form as confirmed by crystal structures showing no hint for the formation of other protein-interacting tungsten species. The stability was tested at the common crystallization temperatures of 4 and 20 °C, respectively, and at TEW concentrations ranging from 1 to 20 mM indicating that the stability is fairly independent of the used concentration. In particular, TEW proved to be stable at pH = 7.5 (used for the crystallization of abPPO₄) at pH = 5.0 (used for the crystallization of cgAUS1) and at pH = 4.8 (used for the crystallization of HEWL). Therefore, it can be surely recommended to use TEW from pH 4.5 to 7.5 in aqueous solution covering a relative wide pH range.

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tissue specific calf intestine alkaline phosphatase and tissue
nonspecific alkaline phosphatase, where TEW showed activity
against tissue nonspecific alkaline phosphatase. Similarly,
chitosan–[(TeW₆O₂₄)₆] was proved to be a potent inhibitor of
calf intestine alkaline phosphatase. However, the exact
binding site and interaction of TEW with the enzyme and thus
the mode of inhibition remains elusive.

3.5. The Negative Net Charge of TEW Ensures Electrostatic
Interaction with the Protein

The total net charge of a POM depends, inter alia, on the
choice of the heteroatom as the higher its oxidation state is the
lower the charge of the complex will be. In the case of TEW
(\([\text{TeW}₆\text{O}₂₄]^{6−}\)), the heteroatom is Te⁶⁺ giving rise to a total net
charge of −6. This high negative charge is predestined for the
interaction with positively charged patches of proteins, and
indeed in all TEW containing crystal structures, TEW was
found at positively charged regions. Thus, TEW should
theoretically target a wide range of proteins as only positively
charged protein surface regions are needed for the interaction.
The TEW–protein interactions are mostly composed of
electrostatic charge–charge interactions (interactions with the
positively charged amino acids lysine and arginine) and H-
bonds. As TEW has a relatively large size, the high negative
charge is distributed over a wide area enabling TEW to
electrostatically interact with large protein portions and
interacting with numerous amino acid residues increasing
both the probability and strength of TEW–protein interactions.
This represents a clear advantage over commonly used (protein
bridging) additives like small molecules or ions carrying a
relatively small charge as they are only able to interact with a
small and limited number of amino acid residues, which in turn
leads to a reduced affinity toward the protein in comparison to
TEW.

3.6. The Size and Shape of TEW Offers Different Variants
of Protein–Protein Bridging

The average dimensions of the Anderson–Evans anion
measures approximately 9 × 9 × 3 (Å³) indicating the
appreciable size of the anion and its planar structure. X-ray
structures of the TEW–protein complexes⁶,⁷,¹²–¹⁵ revealed that
both the size and planar structure are very advantageous during
crystallization. The relatively large size of TEW provides a
certain distance between the protein molecules upon protein–
protein bridging and thus prevents steric interference between
the molecules, which could be a problem when using small
molecules as cross-linking additives (Figure 2). This feature is
even more important when two electrostatically repulsive
protein patches are linked as this TEW-mediated distance could
lead to reduced long-range repulsion forces and at the same
time might increase short-range attraction forces, which are
crucial for the nucleation process.

Due to TEW’s planar structure, this distance can vary
depending on TEW’s orientation leading to bridged mono-
mer–monomer distances in the range of about 6–14 Å (Figure
3). Therefore, different orientations of TEW can induce a
certain versatility in protein–protein bridging, which could
beneficially affect the crystal packing (by offering more freedom
in cross-linking).

3.7. The Symmetry of TEW as a Beneficial Factor for
Protein–Protein Bridging

It was demonstrated that trigonal ([(W₆O₂₄)(O₂CCH₃)₆] with
D₃ symmetry) and pentagonal ([(NaP₄W₁₀O₃₆)]¹⁴− with D₅
symmetry) POMs bind selectively at the crystallographic 3-fold
and 5-fold-axis of the riboflavin synthase structure, respectiv-
ely.⁹ Thus, the symmetry can play a crucial role in POM-
mediated protein–protein cross-linking by selectively directing
the POM binding site and thus its binding behavior. However,
this is only possible if the internal symmetry of the POM
correlates with the protein’s symmetry within the crystal. This
“symmetry-effect” was also observed for TEW during the
crystallization of mushroom tyrosinase abPPO4 where both
TEW anions in the structure were located on the same 2-fold-
axis. The symmetry of TEW is approximately D₃d containing
three C₂ axes and was thus compatible with the crystal’s
symmetry. By directing the binding position of TEW, the
symmetry has also an impact on the degree of protein–protein
cross-linking because TEW being situated on a 2-fold axis
results in an environment where it is surrounded by two protein
molecules, which can be bridged. Therefore, symmetry could
also induce the situation where TEW is located on a
crystallographic 3-fold axis leading to the cross-linking of
three protein molecules. It has to be noted that during the
cocrystallization of both HEWL and cgAUS1 with TEW, the
TEW anions were not exactly or not at all located on

![Figure 2. Schematic illustration of the “electrostatic spacer effect” of
TEW. On the left of the figure, a scenario where three protein patches
(depicted as electrostatic Coulombic surfaces with blue = regions of
positive potential, white = neutral potential, and red = negative
potential) are coming close together is illustrated. This situation can
lead either to steric interference (indicated by a red 10 rays star) or, if
the patches are electrostatically equal, to electrostatic repulsion
(indicated by blue arrows) with both cases leading to no crystal
contacts. However, in the presence of the negatively charged TEW
(illustrated as ball and stick, color code tungsten, cyan; tellurium,
ochre; oxygen, red), the protein patches are electrostatically cross-
linked (indicated by blue and red arrows) and at the same exhibit an
appropriate distance to each other preventing any steric interference.

![Figure 3. Protein–protein bridging by TEW in different orientations.
(A) Two protein molecules (abPPO4) are bridged via TEW lying
vertically between them resulting in a small protein–protein distance.
(B) TEW is positioned horizontally at the interface of two protein
molecules (HEWL) leading to a larger distance between them. The
protein molecules are shown as green cartoons, and TEW is depicted
in ball and stick representation. Color code: carbon, green; nitrogen,
blue; tungsten, cyan; tellurium, ochre; oxygen, red.]

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crystallographic axes indicating a rather random TEW binding; however, the anions still bridged partially more than two protein molecules.

3.8. The Potential of TEW-Mediated Crystallization to Increase Crystal Quality

The ability of TEW to improve the crystal quality and thus the resolution has been observed for cgAUS1, which was crystallized in three crystal forms, two in the absence and one in the presence of TEW.12 All crystal forms were obtained under almost identical crystallization conditions; however, the replacement of MgCl₂ by TEW as crystallization additive increased the resolution dramatically by up to 1.0 Å.14 X-ray structure analysis revealed that the crystal contacts of cgAUS1–TEW are more specific than those of the TEW-less crystal forms, which results in an increase of symmetry and decrease of the number of protein molecules within the asymmetric unit (ASU). All crystal forms were built up of the same crystallographic dimer; however, in the TEW-less structures these dimers formed a tetrameric and octameric arrangement within the ASU, respectively, whereas the ASU of the cgAUS1–TEW structure only contained this crystallographic dimer. In the cgAUS1–TEW structure, two TEW anions mediate new crystal contacts with one TEW strongly stabilizing the crystallographic dimer, which seems to be the reason for the higher crystal quality as the TEW-mediated contacts, especially the dimeric contact, are by far stronger than other rather unspecific contacts within the structure. This leads to a dominating adhesion mode between the proteins (dominated by the TEW-mediated contacts) improving the diffraction behavior of the crystal.45 This is clearly not the case in the TEW-less crystal structures, which lack a preferred adhesion mode and thus exhibit many partially unspecific protein–protein contacts leading to a decreased crystal quality. A similar observation was made in the case of abPPO4. After crystallizing this enzyme in the presence of TEW,6,7 we very recently obtained crystals without TEW but of clearly lower quality (2.76 Å vs 3.25 Å) most likely due to similar reasons as indicated above demonstrating that the TEW-mediated contacts are crucial for crystal quality in those cases.41

3.9. The Ability of TEW To Induce Heterogeneous Crystallization

TEW was reported by our group to mediate “heterogeneous crystal formation”. Mushroom tyrosinase abPPO4 was crystallized in the presence of TEW and resulted in the crystallization of both the latent (64 kDa) and active form (44 kDa) of this enzyme within one single crystal.6 The crystal structures of both forms were unknown until then and with the use of TEW “two birds were killed with one stone”. Each heterodimer (latent and active abPPO4) is on the one side connected to its symmetry mate via an usual protein–protein contact and on the other side linked to the next heterodimer by a TEW-mediated contact composed of two TEW molecules (Figure 4). Two monomers of each abPPO4 share one TEW molecule, which is located on a crystallographic 2-fold axis. This pattern is the structural basis for the entire crystal and demonstrates the possibility to crystallize two protein forms of clearly different size in one single crystal using TEW.

3.10. The Geometric and Functional Flexibility of TEW

In the frequently mentioned cgAUS1–TEW structure, one TEW molecule is unexpectedly covalently bound to the protein leading to the formation of a new TEW-derived cluster with the formula [TeW₆O₂₄O₂(Glu)]⁷⁻ (Figure 5), where the bond is closed between two tungsten atoms and the two carboxylic oxygen atoms (O₂ in the formula) of a glutamic acid (Glu157) bind covalently to two tungsten atoms of TEW accompanied by a rearrangement within the Anderson–Evans structure resulting in a bent structure named GluTEW (illustrated in the left as ball and stick and in the middle as polyhedra). For comparison, the normal Anderson–Evans structure is depicted on the right in a matching orientation as polyhedra. Color code: carbon, green/blue; tungsten, cyan; tellurium, ochre; nitrogen, dark blue; oxygen, red.
cleft, by strong interactions with the surrounding amino acid residues. Therefore, it appears that in this case TEW was able to structurally adapt to the proteinogenic environment in order to fit into the binding cleft. Covalent TEW binding did not alter the overall structure of the protein as indicated by the comparison with TEW-less gAUS1 structures but instead dramatically influenced its crystallization as discussed under reason 3.8. The here provided evidence of the high flexibility of TEW in both geometrical and chemical regards further encourages its use in protein crystallography. The ability to covalently bind to protein residues can lead to the stabilization of flexible protein regions, for example, loops, and thus enhance the crystallizability of proteins suffering from high structural mobility.

4. THE ANDERSON–EVANS POT CAN BE FURTHER TUNED FOR ITS APPLICATION IN PROTEIN CRYSTALLIZATION

We have demonstrated the beneficial effects of TEW as a powerful additive in protein crystallization; however, modifications of the Anderson–Evans structure are possible by (i) changing the central heteroatom resulting in a different net charge, (ii) attaching organic functionalities to the Anderson–Evans core enabling other than electrostatic interactions, and (iii) attaching hydrophobic alkyl chains for potential interaction with membrane proteins as membrane proteins represent the real bottleneck in macromolecular crystallization.

4.1. Tuning of the Net Charge by Selection of the Central Heteroatom

The possibility to tune the total negative net charge of the Anderson–Evans structure by selecting different heteroatoms exhibiting different oxidation states allows the synthesis of clusters with an even higher negative net charge as the one of TEW. The ions Mn4+, Sb5+, Ir5+, and Pt5+ have been incorporated in the Anderson–Evans core as heteroatoms leading to net charges of −7 and −8.10 In general, care should be taken when incorporating metals of lower oxidation states (e.g., Mn3+, Ni3+), as they tend to form the protonated B-type of the Anderson–Evans structure leading to a decreased net charge (−4) in comparison to TEW. An increase in total negative net charge is accompanied by a higher charge density, which could increase the affinity of this derivative toward positively charged proteins.

4.2. Hybridization with Various Organic Functionalities To Target Special Protein Sites

The ability to decorate the inorganic TEW with organic functionalities could be used to synthesize tailor-made Anderson–Evans type structures that could address specific protein sites via their attached organic entity. The Anderson–Evans type polyoxomolydate has explicitly been decorated with a wide variety of tris-ligands (this functionality represents the basis for further modifications) through either pre- or postfunctionalization using different procedures (Figure 6).16,42

The variation in tris-ligands includes alkyl chains of differing lengths, aromatic ligands, ligands with remote binding sites, and ligands with terminated functional groups. Very recently it became possible to tris-functionalize the Anderson–Evans POT core allowing attachment of organic functional groups.22 For example, Anderson–Evans POTs decorated with aromatic ligands could target regions on the protein’s surface with exposed aromatic residues leading to hydrophobic π–π or related stacking interactions, which could support the electro-

Figure 6. Schematic illustration of the synthesis of an organically functionalized Anderson–Evans structure. The TRIS-functionalization step usually takes place at the protonated B-type Anderson–Evans structure (protons are illustrated as white sticks on the left). The resulting TRIS-functionalized structure (in the middle) can then be further functionalized with another organic functionality (indicated as R) leading to a wide range of organic–inorganic hybrid structures (on the right). Color code: carbon, green; addenda atom, cyan; heteroatom and nitrogen, blue; oxygen, red; hydrogen, white.

4.3. Attachment of Large Hydrophobic Moieties on the Anderson–Evans Polyoxotungstate Core Structure Could Lead to the Solubilization of Membrane Proteins and Consequently to Their Crystallization

Furthermore, the attachment of large hydrophobic moieties like long alkyl chains on the Anderson–Evans POT could lead to a POM-based detergent applicable in membrane protein crystallization. Detergents are surface active agents capable of mediating contacts between surfaces differing in polarity, such as hydrophilic and hydrophobic surfaces. They are used in membrane protein crystallography to solubilize the membrane lipid bilayer as most membrane proteins are not soluble in aqueous solutions and thus tend to precipitate due to their hydrophobic domains. Therefore, an Anderson–Evans structure with at least one attached long alkyl chain (in addition to commonly used detergents) could be worth trying in this regard as the hybrid POM could provide valuable protein–protein cross-links (between nonlipid domains) and at the same time stabilize the membrane part of the protein via hydrophobic interactions.

5. OUTLOOK

The application of TEW in the field of protein crystallography will hopefully grow in the future providing crystal structures of proteins for which structures are unknown to this date. The recent successful applications of TEW as crystallization additive suggest that future utilization should bring benefits to several fields like pharmacology, medicine, inorganic chemistry, and especially structural biology, all of them depending on the input from 3D structures. Further systematic investigation of TEW–protein interactions will open new, perhaps today unforeseen, directions.

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The authors declare no competing financial interest.

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ABBREVIATIONS

abPPO4, mushroom tyrosinase 4 (Agaricus bisporus); cgAUS1, aurone synthase from the yellow blossom leaves of Coreopsis grandiflora; GluTEW, TEW covalently bound to a glutamic acid; HEWL, hen-egg white lysozyme; PDB, Protein Data Bank; POMo, polyoxomolybdate; POMs, polyoxometalate; POT, polyoxotungstate; R-Tris, R-C(CH₂OH)₃; TEW, [Te₆W₁₄O₄₄]⁶⁻

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