This is confirmed by the DFT Bader charge of ~+3.7 e in the present compound; in the phosphonates the charge was ~+3.4 e. This difference may be attributed to the presence of an additional oxygen atom in the phosphate group. In the experimental approach the carboxylic O and O(H) oxygen atoms show almost equal charges of 0.92 and 0.97 e respectively, which is not supported by the DFT results - higher on the average by ~0.3 e for these atoms. The phosphate O(H) atom displays a lower or slightly lower charge than the other phosphate oxygen atoms. This is consistent with previous findings [3].
The respective values are -1.43 e for O(H) and -1.46 – 1.67 e for the remaining oxygen atoms in the experimental approach.

The reported topological properties are comparable to those of hydrogen methylphosphonates of calcium and lithium with P-O ρ_c values being near 1.55 $e\cdot Å^{-3}$ and P-O(H) near 1.31 $e\cdot Å^{-3}$. Interestingly the value of ρ_c of P-O(C) bonds is also near to 1.30 $e\cdot Å^{-3}$ as in the P-O(H) bonds.

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Keywords: charge density, phosphoglycolate, phosphorus

L.A.07

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A novel high-throughput approach for purification and reconstitution of large multi-protein complexes

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Proteins are both building blocks and molecular motors for virtually all cellular functions. To perform and regulate complex cellular processes, proteins form interacting heterogeneous assemblies (henceforth referred to as multi-protein complexes or MPCs) whose components exchange continuously during the process. As a result of the transient nature of their interactions, monomeric and multimeric components of MPCs typically have low binding affinities, making their recovery almost impossible during purification. Therefore assembly of MPCs often requires expression and purification of individual (monomeric and multimeric) components in a form (mono-dispersed and properly folded) conducive to reconstitution; furthermore, individual constituents of such large MPCs are frequently insoluble in the absence of the associated components. Thus, identifying conditions leading to MPC reconstitution represents a highly desirable goal in structural biology. To this end we have developed novel solubilization and purification protocols that have allowed us to: 1) solubilize individual monomeric and multimeric components of MPCs; 2) reconstitute (using previously solubilized components), fully active (and stoichiometric) MPCs. Such techniques work equally well on cytosolic and membrane proteins. Despite the intrinsic difficulties that MPCs represent to the field of structural biology, it is crucial to tackle these structural giants since we will only begin to comprehend their biology when the structures of the whole machines have been determined.

Keywords: Biochemistry, Protein, Complex

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Crystallization of a serpin with a insulin-sensitizing function

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Serpins (serine protease inhibitors) are proteins with a molecular mass of about 50 kDa that inhibit chymotrypsin-like serine proteases via the formation of a covalent complex with the target protease. We purified and crystallized SERPINA12, that acts as an adipokine with insulin-sensitizing effects. It could be found in visceral adipose tissue of a rat model of type 2 diabetes. Sequence identity shared with α-1-antitrypsin (41%) suggests a putative serpin function but its molecular target remains to be identified.

To confirm and further analyze the predicted serpin function we intend to determine the three-dimensional structure of the serpin. Thus, His-tagged protein was produced in *Escherichia coli* and purified by Ni-affinity chromatography and size exclusion chromatography. High-troughput screening crystallization trials were prepared in a nanoliter scale with a MicroSys pipetting system using the sitting-drop technique. First diffracting crystals were observed with polyethylene glycol of a molecular weight of 3350 as the precipitant. Crystallization conditions were improved in hanging-drop setups to yield two different crystal forms (space groups $P2_1$ or C2) with similar habitus. C2 crystals show the highest diffraction limit (2.08 Å) and gave rise to a complete dataset collected at BESSY beamline 14.1 at Helmholtz-Zentrum Berlin (Germany).

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Keywords: protein, crystallization

L.A.09

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Crystal structure of bacteriophage T4 fibre protein gp37

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Some viruses and bacteriophages attach to their host cell via specialised fibre proteins, like adenovirus, reovirus and tailed bacteriophages. The bacteriophage T4 short and long fibre proteins, the T5 L-shaped fibres and T7 fibre all have the same basic architecture: they are trimeric and contain an N-terminal phage attachment domain, a long, thin, but stable shaft domain and a more globular C-terminal cell attachment domain. Our goal is to determine the structures of these proteins and thus to make an inventory of stable trimeric folds present in nature.