



PhD Thesis

**Nanostructure Research by Small-Angle X-Ray
Scattering: From Instrument Design to New
Insights into Materials Sciences**

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1 Introduction

Small-angle X-ray scattering (SAXS) is a versatile measurement method for characterizing the structure of matter on the nanometer scale. It has found applications in many different areas of scientific research, ranging from materials to life sciences. Typical use cases are the determination of nanoparticle size-distribution, fractality, supramolecular self-assembling properties, intra- and interchain properties of polymers and polyelectrolytes, low-resolution structure of biological macromolecules, as well as the interactions between them. SAXS is an indirect method inasmuch as the results are not intuitively understandable pictures of the system, thus *a priori* knowledge is frequently indispensable for their interpretation. On the other hand, the results obtainable by this technique are highly accurate and reproducible, because scattering techniques are ensemble methods yielding average quantities on a large number of objects. Furthermore, SAXS experiments are generally non-invasive: samples can be investigated in their native state, usually without special preparation procedures.

While today's high-flux synchrotron lightsources provide ideal grounds for SAXS instruments, a large number of facilities based on laboratory X-ray generators exist worldwide. Although several commercial vendors sell complete, turn-key instruments, unique SAXS cameras have been traditionally constructed in-house, tailored to the needs of the local research group. The largest advantages of local instrumentation, offsetting even the low X-ray flux, are their availability at short notice and their flexibility. Many questions can be answered even with these apparatuses, and in the case of problems not tractable by the local facility, the preliminary SAXS results are still extremely valuable in the planning and successful execution of subsequent synchrotron measurements. Furthermore, *in situ* and *in operando* studies of slow processes can need very long experiment times and frequent or prolonged access to the instrument, which is also an argument

for in-house infrastructure.

SAXS was an important experimental tool in the research group of Dr. Attila Bóta, where I did my research work presented here. The families of materials investigated by this group (activated carbons, phospholipid vesicles, self-assembling nanosystems, etc.) were well-suited for SAXS measurements, and the appropriate experimental apparatus was also available. However, with the fast improvement of instrumentation worldwide, the expected publication standards of SAXS results quickly surpassed its capabilities. Pushing the limits of the instrument eased the situation temporarily.

At the same time the research group has regularly (2-3 weeks on average in each year from 1999 until its decommissioning in 2012) obtained beamtime at the synchrotron instrument B1 (formerly JUSIFA) at the Deutsches Elektronen-Synchrotron (Hamburg, Germany). Since 2006 we were also regular guests at beamline 7T-MPW-SAXS at BESSYII (Berlin, Germany), a possibility continuing to the present day. In 2011 we were awarded measurement time at the beamline ID02 of the European Synchrotron Radiation Facility (Grenoble, France), considered to be the top among SAXS instruments worldwide.

In 2012, funding became available for the group in the form of a joint effort between the Research Centre for Natural Sciences and Gedeon Richter Plc, a prominent pharmaceutical company in Hungary and Europe, for constructing a new in-house SAXS apparatus, based on our experience at the above mentioned large-scale instruments. In designing and realizing the instrument and the corresponding computing infrastructure I assumed the leading role.

2 Objectives

The main goals of my research work were concerned with establishing an up-to-date, state-of-the-art small-angle X-ray scatter-

ing facility in Hungary, which should be able to provide near-synchrotron quality results. This task was two-fold. Firstly, the experimental apparatus had to be designed and built, with the aim to keep its structure simple and highly variable, and to achieve a very high flux and low instrumental background, thus ensuring a flexibility and signal-to-noise ratio uncommon among other laboratory instruments. Secondly, the computing infrastructure had to be installed and developed, for controlling the instrument, acquiring, handling and archiving experimental data. A good experimental practice of SAXS measurement also needed to be implemented at the new facility. In all of these aspects, my previous experience at the above mentioned synchrotron beam-lines turned out to be indispensable. The high intensity, low background, and extreme flexibility of both hardware and software of the resulting instrument is unparalleled in conventional laboratory instruments. The new apparatus has been christened CREDO (standing for Creative Research Equipment for Diffraction).

After finishing most of the construction and programming tasks, the instrument had to be calibrated. In the beginning, I have used already calibrated samples obtained from external sources. Later on, I have done the independent calibration of these samples on CREDO, based on first principles methods.

Validation of the instrument was also important, partly because the interests from the industrial sphere. To achieve this, we took part in an interlaboratory comparison study organized by the Institute for Reference Materials and Measurements of the Joint Research Centre of the European Commission (IRMM-JRC, Geel, Belgium). The aim of this study was to determine the size distribution of SiO₂ nanoparticles using SAXS measured at several independent (synchrotron and laboratory) facilities. One of the most important results of this study was that CREDO has obtained a certificate from IRMM-JRC.

CREDO is a large-scale instrument, capable of more than just to serve the needs of a single research group or institute.

Joining efforts with the small-angle neutron scattering (SANS) instruments of the Budapest Neutron Centre, we have established the Hungarian Small-Angle Scattering Network (HUNSAS) to facilitate access to these experimental apparatuses for guest researchers both from Hungary and abroad, and to ensure a better utilization of the infrastructure. The consortium operates a beamtime proposal system similar to that of synchrotron and neutron facilities. Through this, external researchers can obtain measurement possibilities on CREDO and/or the SANS beamlines simultaneously.

An in-house developed laboratory instrument can never be deemed finished or complete. In order to make the most of the conditions, various aspects of the instrument had to be improved. This included a new method for optimizing the collimating system (with more general results applicable on other small-angle scattering apparatuses), several new sample environments, a complete refurbishment of the instrument control software, and frequent revision of the standard experimental procedures and data assessment/interpretation routines.

Finally, I have illustrated the capabilities of CREDO and SAXS in general by focusing to the following problems:

- characterizing the anisotropic pore structure of activated carbon during the preparation process,
- following the self-assembly of a photoluminescent gold-cysteine nanocomplex *in situ* by time-resolved small-angle X-ray scattering experiments,
- determining the low-resolution structure and folding state of two proteins: hen egg-white lysozyme and human calmodulin.

3 New Scientific Results

I have summarized the results of my doctoral research in the following statements:

1. I have designed and constructed CREDO, a versatile laboratory instrument for small-angle X-ray scattering, capable of providing scattering data with nearly the same quality as obtainable at synchrotron beamlines. A continuous range of the scattering variable ($q = 4\pi \sin \theta / \lambda$) from 0.014 to 29.9 nm⁻¹ is accessible, corresponding to > 400 to 0.2 nm periodic distances. I have also developed a flexible and reliable control software and the accompanying browser-based data processing framework, which covers the tasks of data evaluation, plotting and interpretation, which is rarely found even at large-scale instruments. I have validated the apparatus by accurately deriving the size distribution of SiO₂ nanoparticles, thereby obtaining certification from the Institute of Reference Materials and Measurements of the Joint Research Centre of the European Commission. I have demonstrated the high signal-to-noise ratio and low instrumental background of the instrument by determining the size and shape of two proteins (hen egg-white lysozyme and human calmodulin) from solution SAXS measurements on CREDO, a highly challenging task for traditional laboratory instruments, due to the low scattering power of these samples. I have reported the novel design of this instrument and its other characteristics in [S1]. The homepage of the facility can be found at <http://credo.ttk.mta.hu>. [S1, S2]
2. I have developed a new optimization method for the 3-pinhole collimation scheme, based on algebraically derived results. Defining constraints on the sample and beam-stop size, the set-up yielding the highest radiation intensity at the sample position with negligible instrumen-

tal background can be determined with this method. I have also implemented the results using the `Python` programming language. The resulting proof-of-concept script, `SASCollOpt.py` features a graphical user interface which facilitates its routine usage in the alignment of CREDO, as well as other instruments following the same collimation scheme. [S3]

3. I have executed *in situ* measurements in order to follow the self-assembly of a gold-cysteine nanocomplex using time-resolved small-angle X-ray scattering, and developed automatic model fitting procedures to treat the high amount of experimental data. I have predicted and quantified the evolution of lamellar structures, the existence of which were also proven by transmission electron microscopy. I have found that these lamellae of several nanometer thickness consist of a periodically repeating ensemble of thin layers. I have also characterized the periodic repeat distance and the average number of layers composing the lamellae, as well as the dependence of the speed of self-assembly on the incubation temperature by analyzing the changes in the fitted quantities over the time of incubation. [S4]
4. I studied the changes in the anisotropy of activated carbons introduced by the choice of precursor material and the activation process, based on small-angle X-ray scattering experiments carried out at two different synchrotron beamlines. I have quantified the extent of anisotropy in time (during the activation process), and over the hierarchical structure of the carbon skeleton using azimuthal scattering curves. I have also described pore formation and characterized the typical pore sizes by fitting semi-empirical mathematical models to experimental data. I have also constructed a simple computer model to aid the better understanding of the changes in anisotropy and pore formation observed during

the activation procedure. [S5, S6]

4 Related Publications

- [S1] András Wacha, Zoltán Varga, and Attila Bóta. “CREDO: a new general-purpose laboratory instrument for small-angle X-ray scattering”. In: *J. Appl. Crystallogr.* 47.5 (2014), pp. 1749–1754. ISSN: 1600-5767. DOI: [10.1107/S1600576714019918](https://doi.org/10.1107/S1600576714019918).
- [S2] Attila Bóta, Zoltán Varga, and András Wacha. “Nanoszervezetek meghatározásának új hazai lehetőségei a CREDO kisszögű röntgenszórásos nagyberendezéssel”. In: *Magy. Kémikusok Lapja* 70.10 (2015), pp. 307–311. ISSN: 0025-0163, 1588-1199.
- [S3] András Wacha. “Optimized Pinhole Geometry for Small-Angle Scattering”. In: *J Appl Cryst, J Appl Crystallogr* 48.6 (2015), pp. 1843–1848. ISSN: 1600-5767. DOI: [10.1107/S1600576715018932](https://doi.org/10.1107/S1600576715018932).
- [S4] Balázs Söptei, Judith Mihály, Imola Cs. Szigyártó, András Wacha, Csaba Németh, Imre Bertóti, Zoltán May, Péter Baranyai, István E. Sajó, and Attila Bóta. “The supramolecular chemistry of gold and l-cysteine: Formation of photoluminescent, orange-emitting assemblies with multilayer structure”. In: *Colloids Surf. Physicochem. Eng. Asp.* 470 (2015), pp. 8–14. ISSN: 09277757. DOI: [10.1016/j.colsurfa.2015.01.048](https://doi.org/10.1016/j.colsurfa.2015.01.048).
- [S5] András Wacha, Zoltán Varga, Ulla Vainio, Armin Hoell, and Attila Bóta. “Small-angle X-ray scattering experiments and computer simulations to characterise anisotropy of activated carbons prepared from wood”. In: *Carbon* 49.12 (2011), pp. 3958–3971. ISSN: 00086223. DOI: [10.1016/j.carbon.2011.05.035](https://doi.org/10.1016/j.carbon.2011.05.035).

- [S6] András Wacha, György Onyestyák, and Attila Bóta. “Structure and Sorption Dynamics of Activated Carbons from Wooden Precursors: Further Possibilities to Reveal Anisotropic Behaviors in the Carbon Skeleton”. In: *New Research on Carbon Materials*. Ed. by Cebrian Pesci and Alejandra Ciricillo. Materials Science and Technologies. Hauppauge, NY: Nova Science Publishers, Inc., 2013, pp. 83–112. ISBN: 9781624171871.

5 Other Publications

5.1 Journal Articles

- [J1] Kristóf Bagdi, Kinga Molnár, András Wacha, Attila Bóta, and Béla Pukánszky. “Hierarchical structure of phase-separated segmented polyurethane elastomers and its effect on properties”. In: *Polym. Int.* 60.4 (2011), pp. 529–536. ISSN: 09598103. DOI: [10.1002/pi.3003](https://doi.org/10.1002/pi.3003).
- [J2] Zoltán Varga, András Wacha, Ulla Vainio, J. Gummel, and Attila Bóta. “Characterization of the PEG layer of sterically stabilized liposomes: a SAXS study”. In: *Chemistry and Physics of Lipids* 165.4 (2012), pp. 387–392. ISSN: 0009-3084. DOI: [10.1016/j.chemphyslip.2011.12.011](https://doi.org/10.1016/j.chemphyslip.2011.12.011).
- [J3] Szilvia Berényi, Judith Mihály, András Wacha, Orsolya Tőke, and Attila Bóta. “A mechanistic view of lipid membrane disrupting effect of PAMAM dendrimers”. In: *Colloids Surf. B Biointerfaces* 118 (2014), pp. 164–171. ISSN: 09277765. DOI: [10.1016/j.colsurfb.2014.03.048](https://doi.org/10.1016/j.colsurfb.2014.03.048).
- [J4] Canlan Jiang, Jean-Marie Séquaris, András Wacha, Attila Bóta, Harry Vereecken, and Erwin Klumpp. “Effect of metal oxide on surface area and pore size of water-dispersible colloids from three German silt loam topsoils”. In: *Geoderma* 235-236 (2014), pp. 260–270. ISSN: 00167061. DOI: [10.1016/j.geoderma.2014.07.017](https://doi.org/10.1016/j.geoderma.2014.07.017).

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- [J5] Balázs Söptei, Judith Mihály, Júlia Visy, András Wacha, and Attila Bóta. “Intercalation of Bovine Serum Albumin Coated Gold Clusters Between Phospholipid Bilayers: Temperature-Dependent Behavior of Lipid-AuQC@BSA Assemblies with Red Emission and Superlattice Structure”. In: *J. Phys. Chem. B* 118.14 (2014), pp. 3887–3892. ISSN: 1520-6106. DOI: [10.1021/jp4124138](https://doi.org/10.1021/jp4124138).
- [J6] Zoltán Varga, András Wacha, and Attila Bóta. “Osmotic shrinkage of sterically stabilized liposomes as revealed by time-resolved small-angle X-ray scattering”. In: *J. Appl. Crystallogr.* 47.1 (2014), pp. 35–40. ISSN: 1600-5767. DOI: [10.1107/S1600576713030513](https://doi.org/10.1107/S1600576713030513).
- [J7] Róbert Deák, Judith Mihály, Imola Cs. Szigyártó, András Wacha, Gábor Lelkes, and Attila Bóta. “Physicochemical characterization of artificial nanoerythrocytes derived from erythrocyte ghost membranes”. In: *Colloids and Surfaces B: Biointerfaces* 135 (2015), pp. 225–234. ISSN: 0927-7765. DOI: [10.1016/j.colsurfb.2015.07.066](https://doi.org/10.1016/j.colsurfb.2015.07.066).
- [J8] András Lőrincz, Judith Mihály, Csaba Németh, András Wacha, and Attila Bóta. “Effects of ursolic acid on the structural and morphological behaviours of dipalmitoyl lecithin vesicles”. In: *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1848.5 (2015), pp. 1092–1098. ISSN: 0005-2736. DOI: [10.1016/j.bbamem.2015.01.010](https://doi.org/10.1016/j.bbamem.2015.01.010).
- [J9] Lívia Naszályi Nagy, Judith Mihály, András Polyák, Balázs Debreczeni, Barbara Császár, Imola Csilla Szigyártó, András Wacha, Zsuzsanna Czégény, Emma Jakab, Szilvia Klébert, Eszter Drotár, G Dabasi, Attila Bóta, Lajos Balogh, and Éva Kiss. “Inherently fluorescent and porous zirconia colloids: preparation, characterization and drug adsorption studies”. In: *J. Mater. Chem. B* 3.38 (2015), pp. 7529–7537. ISSN: 2050-750X, 2050-7518. DOI: [10.1039/C5TB00832H](https://doi.org/10.1039/C5TB00832H).

- [J10] Ákos Szabó, András Wacha, Ralf Thomann, Györgyi Szarka, Attila Bóta, and Béla Iván. “Synthesis of Poly(methyl methacrylate)-poly(poly(ethylene glycol) methacrylate)-polyisobutylene ABCBA Pentablock Copolymers by Combining Quasiliving Carbocationic and Atom Transfer Radical Polymerizations and Characterization Thereof”. In: *J. Macromol. Sci. Part A* 52.4 (2015), pp. 252–259. ISSN: 1060-1325, 1520-5738. DOI: [10.1080/10601325.2015.1007268](https://doi.org/10.1080/10601325.2015.1007268).
- [J11] J. Carlos Almeida, A. Wacha, A. Bóta, L. Almásy, M. Helena Vaz Fernandes, Fernanda M. A. Margaça, and Isabel M. Miranda Salvado. “PDMS-SiO₂ hybrid materials – A new insight into the role of Ti and Zr as additives”. In: *Polymer* 72 (2015), pp. 40–51. ISSN: 0032-3861. DOI: [10.1016/j.polymer.2015.06.053](https://doi.org/10.1016/j.polymer.2015.06.053).
- [J12] J. Carlos Almeida, András Wacha, Pedro S. Gomes, Luís C. Alves, M. Helena Vaz Fernandes, Isabel M. Miranda Salvado, and M. Helena R. Fernandes. “A biocompatible hybrid material with simultaneous calcium and strontium release capability for bone tissue repair”. In: *Materials Science and Engineering: C* (2016). ISSN: 09284931. DOI: [10.1016/j.msec.2016.01.083](https://doi.org/10.1016/j.msec.2016.01.083).

5.2 Hungarian-language Articles

- [O1] Lívia Nagyné Naszályi, Marcell Pálmai, Adrienn Pethő, Barbara Császár, András Polyák, Imola Csilla Szigyártó, Judith Mihály, András Ferenc Wacha, and András Lőrincz. “Szervetlen gyógyszerhordozó nanorészecskék előállítás és jellemzése”. In: *Magy. Kémiai F. - Kémiai Közlemények* 121.2-3 (2015), pp. 110–116.
- [O2] Ákos Szabó, Péter Mezey, Csaba Fodor, Attila Domján, Gergely Kali, Tímea Stumphauer, Gábor Erdődi, Ralf Thomann, Péter Németh, István Szanka, Gergely Illés,

Márton Haraszti, Szabolcs Pásztor, Attila Bóta, András Wacha, Károly Süvegh, and Béla Iván. “Amfífil polimer kotérhálók: egy újszerű nanoszerkezetű anyagcsoport”. In: *Magy. Kémiai F. - Kémiai Közlemények* 121.2-3 (2015), pp. 89–93.

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