APPLICATION NOTE:
Gd-HPDO$_3$A complex for CV-SAXS experiments

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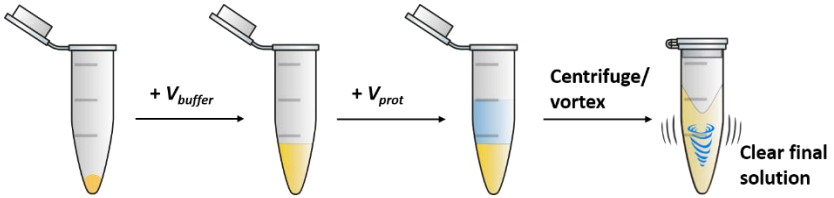
Gd-HPDO$_3$A, an efficient and inert gadolinium-based contrast agent to perform contrast variation SAXS experiments (CV-SAXS) for a broad range of biological systems such as detergent micelles, proteins, protein/nucleic acid complexes, has been previously tested[1,2]. In practice, the gadolinium complex will generate contrast variation by replacing 40 to 50% of the solvent. Gd-HPDO$_3$A is supplied as a white powder in microtubes having a conical bottom and screw cap in ready to use kits: **CV-SAXS A/** and **B/**.

Prior to performing the full CV-SAXS experiment, two preliminary analyses are strongly recommended: **Protein concentration variation** and **Experiments with low concentrations of Gd-HPDO$_3$A (A/ Primo test)** (To check protein stability in the presence of contrast agents).

<u>Preparation of the Gd-complex concentration ranges:</u>

Gd-HPDO$_3$A solution preparation are calculated for a final fixed volume of **50 µL**, which can be used in most synchrotron beamlines with batch experimental setups. Each experiment should be performed with the protein buffer with and without the protein (with the previously identified fixed final protein concentration). It is crucial to use the same buffer for all experiments. A minimum protein concentration of 5 mg/ml is recommended to obtain adequate scattering intensity. Concentration and contrast variation SAXS experiments must be performed on the same beamline with identical parameters.

Protocol:



1- Define the **optimal protein concentration C_{SAXS}** by conventional SAXS experiments: evaluate protein stability in buffer over a concentration range of at least 1 to 10 mg/mL (in aqueous buffer in the absence of Gd-HPDO$_3$A) to determine the best concentration with the highest stability for the following steps.
   A constant protein concentration C_{SAXS}, in a 50 µL volume, must be maintained along the series. **Calculate the volume of protein solution: $V_{protein} = \frac{C_{SAXS} \times 50\ \mu L}{C_{protein}}$,** required to reach this concentration ($C_{protein}$ = protein concentration of the stock solution, which has to be at least a factor 1.7 compared to C_{SAXS}). **Attention! The presence of large amounts of the gadolinium complex occupies a non-negligible volume in the final solution. Thus, $V_{protein}$ must be lower than 29.4 µl to allow the highest Gd-HPDO$_3$A concentration.**
2- The gadolinium complex Gd-HPDO$_3$A needs to be dissolved with the correct amount to reach precisely the final volume of 50 µl so it is also essential to respect the calculated additional buffer volume V_{buffer}.
3- Subtract the protein volume $V_{protein}$ from the maximum buffer volume V_{buffer} corresponding to each Gd concentration according to the table below.
4- It can take time for the complex powder to dissolve after adding the protein and/or buffer solution. Do not hesitate to alternate several rounds of vortexing and centrifuging at 10,000 rpm for 2 minutes of the samples for complete homogeneity.
5- To obtain the final solutions, ensure complete dissolution of all components. Visual inspection is crucial at this stage; employing a lamp can aid in identifying any aggregates or undissolved components. If necessary, repeat step 5 to ensure thorough dissolution.
6- The final solutions must be very homogeneous. If this is not the case (milky, turbid aspect, …), it is probably an aggregation and there is no interest in continuing the experiment.

Preparation of the Gd-complex ranges:

A/ Primo test (0 to 0.4M of Gd): This first range of low Gd-$HPDO_3A$ concentrations allows to perform first trials to check the compatibility and the stability of the protein in the presence of the molecule.

A1 – Protein samples in buffer + $GdHPDO_3A$

Samples (vial name)	**1 (R)**	**2 (A)**	**3 (B)**	**4 (C)**
Mass of gadolinium complex (mg)	0	2.8	5.6	11.3
Volume of protein (µL) = $V_{protein}$	$V_{protein}$	$V_{protein}$	$V_{protein}$	$V_{protein}$
Volume of buffer (µL) = V_{buffer}	$V_{buffer} = 50 - V_{protein}$	$V_{buffer} = 48.3 - V_{protein}$	$V_{buffer} = 46.6 - V_{protein}$	$V_{buffer} = 43.1 - V_{protein}$
Final concentration of Gd complex (M)	**0**	**0.1**	**0.2**	**0.4**

A2 – Buffer only + $GdHPDO_3A$

Samples (vial name)	**1 (R)**	**2 (A)**	**3 (B)**	**4 (C)**
Mass of gadolinium complex (mg)	0	2.8	5.6	11.3
Volume of protein (µL) = $V_{protein}$	0	0	0	0
Volume of buffer (µL) = V_{buffer}	50	48.3	46.6	43.1
Final concentration of Gd complex (M)	**0**	**0.1**	**0.2**	**0.4**

B/ Full CV-SAXS experiment:

B1 – Protein samples in buffer + $GdHPDO_3A$

Samples (vial name)	**1 (R)**	**2 (D)**	**3 (E)**	**4 (F)**	**5 (G)**	**6 (H)**
Mass of gadolinium complex (mg)	0	3.5	7	14.1	21.1	33.8
Volume of protein (µL) = $V_{protein}$	$V_{protein}$	$V_{protein}$	$V_{protein}$	$V_{protein}$	$V_{protein}$	$V_{protein}$
Volume of buffer (µL) = V_{buffer}	$V_{buffer} = 50 - V_{protein}$	$V_{buffer} = 47.9 - V_{protein}$	$V_{buffer} = 45.7 - V_{protein}$	$V_{buffer} = 41.4 - V_{protein}$	$V_{buffer} = 37.1 - V_{protein}$	$V_{buffer} = 29.4 - V_{protein}$
Final concentration of Gd complex (M)	**0**	**0.125**	**0.250**	**0.500**	**0.750**	**1.2**

B2 – Buffer only + $GdHPDO_3A$

Samples (vial name)	**1 (R)**	**2 (D)**	**3 (E)**	**4 (F)**	**5 (G)**	**6 (H)**
Mass of gadolinium complex (mg)	0	3.5	7	14.1	21.1	33.8
Volume of protein (µL) = $V_{protein}$	0	0	0	0	0	0
Volume of buffer (µL) = V_{buffer}	50	47.9	45.7	41.4	37.1	29.4
Final concentration of Gd complex (M)	**0**	**0.125**	**0.250**	**0.500**	**0.750**	**1.2**

Attention, the presence of large amounts of the gadolinium complex occupies a non-negligible volume in the final solution. For this reason, it is essential to respect the maximum buffer volume indicated to reach the final volume of 50 µl. The highest concentration of Gd-$HPDO_3A$ used is 1.2 M which corresponds to a saturated solution of gadolinium complex.

References:

[1]Gabel, F., Engilberge, S., Schmitt, E., Thureau, A., Mechulam, Y., Pérez, J., & Girard, E. (2022). Medical contrast agents as promising tools for biomacromolecular SAXS experiments. Acta Cryst., D78, 1120-1130.

[2]Gabel, F., Engilberge, S., Pérez, J., & Girard, E. (2019). Medical contrast media as possible tools for SAXS contrast variation. IUCrJ 6 (4), 521-525