Supporting Information to

Self-confined nucleation of iron oxide nanoparticles
in a nanostructured amorphous precursor

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Materials and methods

Chemicals and synthetic procedure

Ferrous chloride tetrahydrate (FeCl$_2$·4 H$_2$O), ferric chloride hexahydrate (FeCl$_3$·6 H$_2$O), hydrochloric acid (HCl, 1 M) and sodium hydroxide analytical concentrate (NaOH, 5 M) were purchased from Sigma-Aldrich. Dilutions of HCl and NaOH were prepared with deionized water. To avoid oxidation of ferrous iron, Fe(II) and Fe(III) chloride were dissolved in 1:2 stoichiometry in 100 mM HCl, resulting in solutions with total [Fe] ranging from 10 to 100 mM. Reactions were carried out by injecting equal volumes of the iron chloride solutions and freshly prepared 1 M NaOH into either a reaction vessel (15 mL each) via a custom-made millifluidic mixer at a total flow rate of 60 mL min$^{-1}$, or the reaction chamber of a stopped-flow setup (Biologic).

Determination of dissolved iron at reaction end

Ferrous and ferric iron chloride solutions (5 mL, total [Fe] = 10 – 100 mM with 1 to 2 stoichiometry) were rapidly mixed with sodium hydroxide (5 mL, 1 M). To obtain solutions containing only dissolved Fe, the products were filtered after 10 min incubation using cellulose acetate syringe filters with 0.2 µm pore size (Whatman FP30/0.2 CA-S). After acid digestion with concentrated nitric acid, the solutions were analyzed by ICP-OES (Optima 8000, Perkin Elmer) using the Software WinLab 5.1. The device was calibrated using a multi-element standard solution 28 (2650.1) by Roth.
Small and wide angle X-ray scattering (SAXS/WAXS) data acquisition

Time-resolved X-ray scattering patterns have been recorded on the SWING beamline of the SOLEIL synchrotron (Saint-Aubin, France) with a photon energy of 12.5 keV.\textsuperscript{1} In configuration #1, the reactive mixture circulated in a Kapton capillary (3mm diameter); each experiment was carried out twice, in SAXS (sample–detector distance of 6.3 m, acquisition times of 50-500ms), and in WAXS (58 cm, 1250-2000ms acquisition time). Superimposition of both datasets leads to a full range of $q = 5.6 \times 10^{-3}$ Å\textsuperscript{-1} to 3.1 Å\textsuperscript{-1} ($q = 4\pi/\lambda \sin \theta$, with $2\theta$ the angle between the incoming and scattered beam), with an overlap of c.a. 400 SAXS and WAXS data points in the $q = 1.86 \times 10^{-2}$ to 0.44 Å\textsuperscript{-1} range, with an accuracy better than 5%. The accessible reaction times were $7s < t < 600s$ every 3s. In configuration #2, the reaction was carried out in a commercial stopped-flow setup (Biologic) to reach smaller dead times. The sample-detector distance was 6.4 m with acquisition times of 20 ms, leading to a range of $q = 5.3 \times 10^{-3}$ Å\textsuperscript{-1} to 0.44 Å\textsuperscript{-1}, accessible reaction times of $150 \text{ ms} < t < 300 \text{ s}$ (limited by sedimentation) with a time resolution of 200 ms.

All intensities are expressed in differential cross-section per volume unit:

$$I(q) = \frac{dN(q)}{N_{tot}} \frac{1}{\Delta\Omega \delta x}$$

where $\delta x$ is the thickness of the sample, $dN(q)/N_{tot}$ is the probability for a transmitted photon to be scattered with a momentum transfer $q$ and collected in the solid angle $\Delta\Omega$. $dN(q)$ was measured after azimuthal integration of signal collected on the detector (Dectris Eiger X 4M), and $N_{tot}$ was estimated from the beam transmitted to a photodiode mounted after the sample. The detector and photodiode were calibrated using water as a reference scatterer (1.62 $10^{-2}$ cm\textsuperscript{-1}), and water is subtracted from all normalized scattering patterns.
Data analysis

SAXS/WAXS data analysis and comparison to the nucleation theory was performed using a custom Python library as follows:

i) Retrieval of the total volume fraction of amorphous + crystalline materials $\phi_{\text{tot}}$:

$\phi_{\text{tot}}$ is calculated using the invariant theorem:

$$\int_{0}^{+\infty} I(q)q^2dq = 2\pi^2 \phi_{\text{tot}}(1 - \phi_{\text{tot}}) \Delta \rho^2$$

where $\Delta \rho$ is the scattering length density contrast between water and the condensed materials, assumed equal for magnetite and the amorphous aggregates. In order to extrapolate data to $q \to 0$ and $q \to +\infty$ and integrate, the following equation has been fitted to the data (see vii below):

$$I(q) = B_0 q^{-p_o} \exp \left( - \frac{q^2 R_1^2}{3} \right) + G_1 \exp \left( - \frac{q^2 R_2^2}{3} \right) + B_1 (q_1^4)^{-1} + G_2 \exp \left( - \frac{q^2 R_2^2}{3} \right) + I_\infty$$

where the three first terms originate from the Beaucage unified model which describes properly aggregates of primary particles; the fourth term accounts for a constant residual signal at large q values (sizes of a few Angstroms) assigned to the form factor of hydrated ions; and the last term is the constant incoherent scattering of all ions.

The condensed (amorphous+crystal) volume fraction is obtained after numerical integration of the relevant part of the signal, i.e.:

$$2\pi^2 \phi_{\text{tot}}(1 - \phi_{\text{tot}}) \Delta \rho^2 = \int_{0}^{+\infty} \left( B_0 q^{-p_o} \exp \left( - \frac{q^2 R_1^2}{3} \right) + G_1 \exp \left( - \frac{q^2 R_2^2}{3} \right) + B_1 (q_1^4)^{-1} \right) q^2 dq$$

$2\pi^2 \phi_{\text{tot}}(1 - \phi_{\text{tot}})$ is found constant and equal within 10% to the value expected from the nominal concentrations and electron density of the crystal.

This approach assumes that the scattering law decays as $I(q) \propto q^{-4}$ (Porod’s law) above a cut-off value of $q \approx 1/R$. We have checked that the invariant depends very little (less than 5%) on the
choice of the cut-off value in the range 0.7-1 Å\(^{-1}\). This indicates that the experimental signal, prior to extrapolation, is already close to a Porod’s law.

ii) Retrieval of the crystalline volume fraction \(\phi_{\text{cry}}\):

\(\phi_{\text{cry}}\) is taken proportional to the (311) Bragg peak area obtained after fitting a pseudo-Voigt function to the signal in the the 2.3 Å\(^{-1}\) < \(q\) < 2.7 Å\(^{-1}\) region. The proportion factor is found by assuming a complete yield after 10 minutes of reaction at 50 mM (Fig. 2A).

iii) Retrieval of the SAXS contribution of the crystalline phase:

We assume that the total SAXS signal is the sum of the crystalline and amorphous contributions. The latter contribution is subtracted assuming that only the amount of amorphous aggregate changes with time, not its structure. Hence, at any reaction time \(t\):

\[
l_{\text{cry}}(t) = I(t) - \left( 1 - \frac{\phi_{\text{cry}}(t)}{\phi_{\text{tot}}} \right) I_{\text{amorph}}(t_0)
\]

where we choose a reference reaction time \(t_0\) for which \(\phi_{\text{cry}}(t_0) = 0\), and we assume \(\phi_{\text{amorph}}(t) = \phi_{\text{tot}} - \phi_{\text{cry}}(t)\).

iv) Model fitting to the crystalline signal:

In order to retrieve from \(l_{\text{cry}}\) the structural data characteristic of the crystalline part, we used a model describing the aggregation of crystals in coexistence with ions in solution, and a residual constant background:

\[
l_{\text{cry}}(t) = S_{\text{cry}}(q) P_{\text{cry}}(q) + P_{\text{ions}} + I_{\text{back}}
\]

with \(P_i\) the phenomenological form factor of Beaucage:
\[ P_i(q) = G_i \exp\left(-\frac{q^2 R_i^2}{3}\right) + B_i(q_i^*)^{-4} \]

and \( S_{\text{cryst}} \) is the structure factor describing the aggregation of the crystals into mass fractal particles of size \( \xi \) and fractal dimension \( 1 \leq D < 3 \):

\[ S_{\text{cryst}}(q) = 1 + \frac{D \Gamma(D - 1) \sin((D - 1)\tan(q \xi))}{(q R_{\text{cryst}})^D (1 + (q \xi)^{-2})^{D-1}} \]

In practice, we found the best fits using an aggregation size \( \xi \to +\infty \), yielding the usual expression for fractal networks of size larger than \( 1/q_{\text{min}} \).

v) Analysis of the fit parameters:

We have translated into structural parameters the fit results of \( I_{\text{cryst}} \):

- \( \phi_{\text{cryst}} \), the crystalline volume fraction, has been evaluated by numerical integration of the model:
  \[ \int_0^{+\infty} I_{\text{cryst}}(q) q^2 dq = 2\pi^2 \phi_{\text{cryst}} (1 - \phi_{\text{cryst}}) \Delta \rho^2 \]. We find a good agreement with the crystalline volume fraction evaluated from the integration of the Bragg peak, which validates the retrieval of the crystals scattering signal.

- \( R \), the radius of the crystalline particles, has been calculated from the radius of gyration \( R_1 \) assuming spherical, dense objects:
  \( R = \sqrt{5/3} R_1 \)

- \( v_{\text{cryst}} \), the volume of the crystals, is retrieved from the crystal volume fraction and a general relation with the \( q = 0 \) value of the form factor:
  \( v_{\text{cryst}} = \frac{g_{\text{cryst}}}{\phi_{\text{cryst}} \Delta \rho^2} \)

- \( n \), the numerical concentration of primary particles, has been calculated after elimination of the volume of the crystals from \( P_{\text{cryst}}(0) = G_{\text{cryst}} \cdot \bar{n} = \frac{\phi_{\text{cryst}} \Delta \rho^2}{g_{\text{cryst}}} \)
vi) Comparison of experimental and theoretical nucleation rates

$$J^{st},$$ the steady-state nucleation rate, was extracted from experimental $\bar{n}$ with a linear regression between reaction times of 30s to 75% of the reaction time where $\bar{n}$ is maximal.

To compare the experimental rates with predictions from classical kinetic theories, which provide steady-state nucleation rates of particles that grow or dissolve by monomers, we assume the result still holds for ionic crystals, considering they grow and dissolve by ion pairs or particles. The work of formation $W(N)$ of a spherical particle of size $N$ is given by $W(N) = -|g|N + \sigma A - W_{ref},$ where $g$ is the free energy of formation for the crystal per monomer, $\sigma$ the interfacial tension between the crystal and solvent, $A$ the surface area and $W_{ref}$ an offset ensuring null work of formation for the monomer ($W(1) = 0$).

Growth and dissolution rates balance out at the critical particle size $N^* = -\frac{32\pi v^2\sigma^3}{3|g|^3},$ where $v$ is the monomer volume. An approximate analytical expression for the steady-state nucleation rate $J^{st}$ is determined by the monomer attachment rate at the critical size $k^+(N^*),$ and the steady-state particle population at the critical size $\bar{n}(N^*).$ The latter deviates from the usual thermodynamic Boltzmann distribution $\bar{n}(N^*)$ by the Zeldovich regression factor $Z(N^*) = \left(\frac{|g|}{6\pi kT N^*}\right)^{1/2},$ which accounts for population depletion by growth and dissolution, giving finally:

$$J^{st} = k^+(N^*)Z(N^*)\bar{n}(N^*)$$

$J^{st}$ was evaluated assuming either crystallization from ions, or from the dense amorphous particles, using the following intermediate expressions:

- critical size $N^* = \frac{32\pi v^2\sigma^3}{3|g|^3}$

- the Zeldovich factor $Z(N^*) = \left(\frac{|g|}{6\pi kT N^*}\right)^{1/2}$
- the equilibrium particle size distribution at the critical size,
\[ \tilde{n}^eq(N^*) = \tilde{n}^eq(1) \exp\left(-\frac{W(N^*)}{kT}\right) \]

- for crystallization from the ions in solution (direct crystallization or dissolution-recrystallization), the free energy of reaction per Fe monomer is written for the reaction:
\[ 2 \text{Fe}^{3+}(\text{OH})_a + \text{Fe}^{2+}(\text{OH})_b + (8-2a-b) \text{OH}^- \rightarrow \text{Fe}_3\text{O}_4 + 4 \text{H}_2\text{O} \]

It gives:
\[ g = g_{\text{ions-cryst}}^0 - \frac{kT}{3} \ln\left(\frac{4^3}{3} c_{Fe}\right) - \frac{8 - 2a - b}{3} kT \ln\left(c_{OH^-} - \frac{2a + b}{3} c_{Fe}\right) \]

- for crystallization from ions in solution, we take for \( k^+ \) the rate of attachment as calculated by the equation of Smoluchowski: the resolution of Fick’s equation for spherically symmetric diffusion across a sphere of radius \( R \) of monomers of initial concentration \( c_0 \) and diffusion coefficient \( D \) gives a rate of attachment of:
\[ k_{\text{Smolu}} = 4\pi D R c_0 \]

In the case of mutual aggregation of two diffusing particles, the rate of attachment is calculated using the sum of diffusion coefficients and radii, leading for identical particles to:\(^6\)
\[ k^+ = 16\pi D R c_0 \]

- for crystallization from the amorphous particles, the free energy of reaction per Fe monomer is assumed in the \( |g| \geq 5kT \) range.

vii) Fitting procedure

Where needed, the models were fitted to the data using a least-square Levenberg-Marquardt algorithm using the data standard deviation to weigh the \( \chi^2 \) function. To avoid trapping in local
minima, each fit was reproduced 30 times after varying the initial parameters by 10%. The retained best fit is the set of parameters with both the lowest $\chi^2$ and a non-singular covariance matrix.

viii) Evaluation of the confidence intervals

To estimate the standard deviation on all fit and physical parameters, the 95% confidence interval for the $i$-th parameter was evaluated by the resampling (or “bootstrapping”) method: $N=100$ $(I, q)$ datasets were randomly generated where each $I(q)$ data point is normally distributed around the experimental point with a variance equal to the experimental standard deviation. After making the complete analysis of each of the $N$ datasets (up to the nucleation rates, interfacial tensions etc.), we made the histograms of the parameters. We found that the 95% confidence interval of all fit parameters physical parameters is within ±5% the mean value. For clarity, a Gaussian function has been fitted to the histograms and only the standard deviation is presented in the text.

ix) Code availability

The custom code – written in Python 2.7 – that support the findings of this study are available from the corresponding authors on request.
Transmission electron microscopy

Particles were adsorbed to carbon film-coated Cu mesh grids by surface immersion with the reactant suspension. To remove salt precipitates, grids were carefully rinsed with deionized water and dried with Kimwipe paper. Imaging was performed on a Zeiss EM 912 Omega with 120 kV acceleration voltage.
X-ray powder diffraction

To assess the phase of the produced crystalline material and determine grain sizes, we performed X-ray diffraction of deionized H$_2$O washed and dried precipitates of the reaction products after 10 min reaction time at µ-Spot beamline, BESSY II, Berlin at 15 keV. Sample-detector distance was calibrated using a quartz standard (NIST 1878b) and diffraction patterns were recorded on a Dectris EIGER X 9M detector. We determined the lattice constant $a$ of the magnetite unit cell with $Fd\bar{3}m$ symmetry using the relation $a = \frac{2\pi}{q_{hkl}} \sqrt{h^2 + k^2 + l^2}$, where $q_{hkl}$ is the fitted position of the peak with Miller indices $hkl$. Fits were performed with Origin 8.6 using a Pseudo-Voigt profile on the Bragg reflections (111), (220), (311), (400), (422), (511) and (440) to determine the error-weighted mean lattice constant $a$ and the coherence length $d = \frac{2\pi}{FWHM}$, i.e. the particle diameter when neglecting strain contributions.

**Table SI1.** Lattice constant, particle size and solubility of magnetite nanoparticles formed as function of the total iron concentration.

<table>
<thead>
<tr>
<th>Total [Fe] (mM)</th>
<th>Lattice constant $a$ (nm)</th>
<th>Volume-averaged particle size $d$ (nm)</th>
<th>Solubility [Fe] (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.83955±0.00002</td>
<td>6.2±0.5</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>37.5</td>
<td>0.83977±0.00003</td>
<td>5.7±1.1</td>
<td>0.18±0.04</td>
</tr>
<tr>
<td>25</td>
<td>0.83972±0.00002</td>
<td>5.0±0.6</td>
<td>0.26±0.06</td>
</tr>
<tr>
<td>17.5</td>
<td>0.83994±0.00007</td>
<td>4.7±0.5</td>
<td>0.45±0.09</td>
</tr>
<tr>
<td>10</td>
<td>0.84112±0.00012</td>
<td>3.0±0.7</td>
<td>1.26±0.38</td>
</tr>
<tr>
<td>7.5</td>
<td>n.d.</td>
<td>2.9±0.5</td>
<td>2.30±0.73</td>
</tr>
<tr>
<td>5</td>
<td>0.83856±0.00023</td>
<td>2.1±0.3</td>
<td>3.97±0.67</td>
</tr>
</tbody>
</table>
The diffraction patterns of magnetite and maghemite are very similar, yet can be distinguished by the lattice constant. The determined lattice constants are consistent with magnetite (bulk magnetite is reported as 0.8397 nm,\(^8\) while maghemite as 0.8334 nm\(^9\)).

**Figure S11.** Powder diffraction of material formed at [Fe] = 50 mM and Bragg peak fits.
Size-dependent solubility of formed magnetite nanoparticles

The solubility of iron oxide nanoparticles

\[
\log [\text{Fe}] = \log K + \frac{2}{3} \frac{\bar{\gamma} M \alpha}{2.3RT \rho d}
\]

is given by their bulk solubility product \( \log K \) and a surface contribution \( \frac{2}{3} \frac{\bar{\gamma} M \alpha}{2.3RT \rho d} \) where \( \bar{\gamma} \) is the mean surface tension, \( M \) is molecular weight, \( \alpha \) is a geometric shape factor related to the surface-to-volume ratio, \( R \) is the gas constant, \( T \) is temperature, \( \rho \) is density and \( d \) is the particle size.\textsuperscript{10}

Hence a linear fit of solubilities \( \log [\text{Fe}] \) over the inverse particle size as measured by SAXS can reveal the mean surface tension \( \bar{\gamma} \) and the bulk solubility \( \log K \) (at the prevalent pH and ionic strength). From the fit we obtain \( \log K = -7.5 \) and \( \frac{2}{3} \frac{\bar{\gamma} M \alpha}{2.3RT \rho} = 5 \times 10^{-9} \text{ m}^{-1} \) (Fig. S12). Assuming \( M = 231.533 \text{ g/mol} \) and \( \rho = 5.175 \times 10^6 \text{ g/m}^3 \) for magnetite and assuming spherical particles \( \alpha = 6 \), we can infer a mean surface tension \( \bar{\gamma} = 0.16 \text{ J m}^{-2} \).
Figure S12. Magnetite nanoparticle solubility as a function of inverse diameter reveals bulk solubility and size-dependent contributions from which a mean surface tension can be inferred.
SAXS data from a stopped-flow configuration

**Figure S13.** Radius of gyration $R_g$ (black) and crystalline volume fraction $\phi_{\text{cryst}}$ (red) over time for the crystallization of magnetite nanoparticles at a total iron concentration $c(\text{Fe}) = 50$ mM. Size data were obtained using different experimental setups: fast continuous flow (black triangles), stop-flow (black circles) and slow continuous flow (black squares). Total volume fraction of crystalline phase was determined independently from Bragg peak integration (triangles) and SAXS analysis (squares).
Deconvolution of the crystals contribution to the SAXS signal

Figure SI4. Crystalline contribution to the SAXS signal over time for a $c = 37.5$ mM synthesis.
Figure SI5. Transmission electron micrographs of dried particle aggregates forming at different total iron concentrations (yellow). Insets: Small area electron diffraction with indexed reflections (magnetite). Below 17.5 mM total Fe crystallization did not occur.
Additional references


