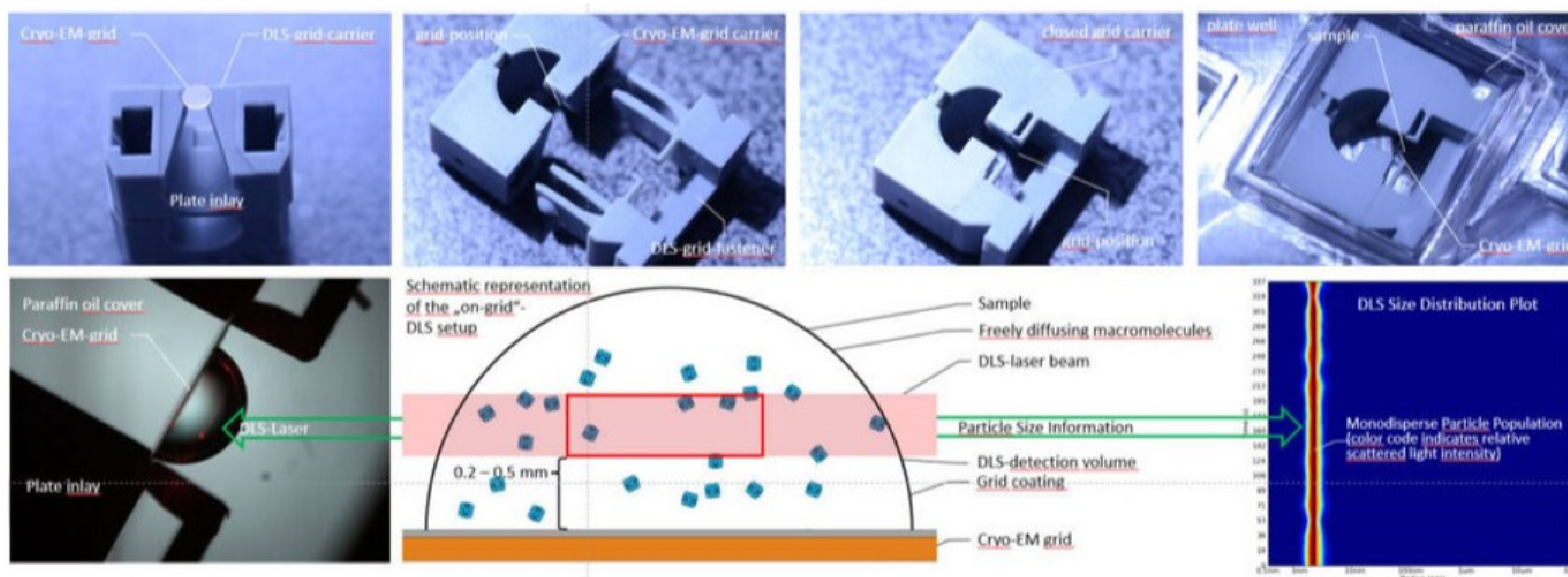
Xtal Concepts

Newsletter

Evaluation of Protein Stability on Cryo-EM Grids by DLS

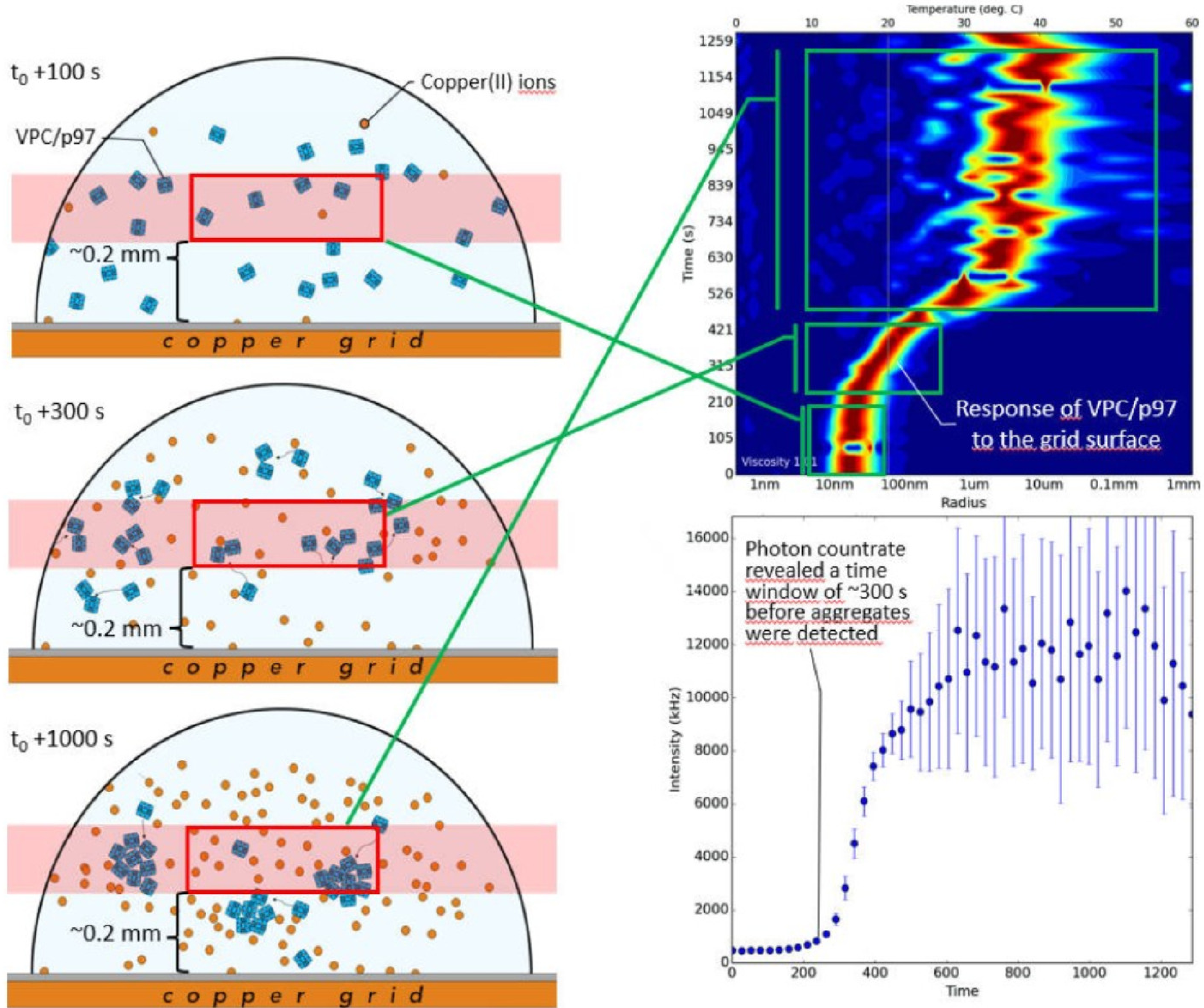
DLS measurements can be performed directly on cryo-EM grids by placing a sample-loaded cryo-EM grid in a specially designed plate for DLS. This "on-grid" DLS is the very last analytical step in the single-particle 3D cryo-EM sample preparation process where information can be obtained. The response of a sample after contact with a cryo-EM grid is often remarkable. These investigations are made possible by the unique optical arrangement of the SpectroLight 600 DLS optics, which underlines the versatility of the system. This method will help to increase the success rate of future cryo-EM approaches.

Grid Support to maintain parallel Alignment with the DLS Laser Beam

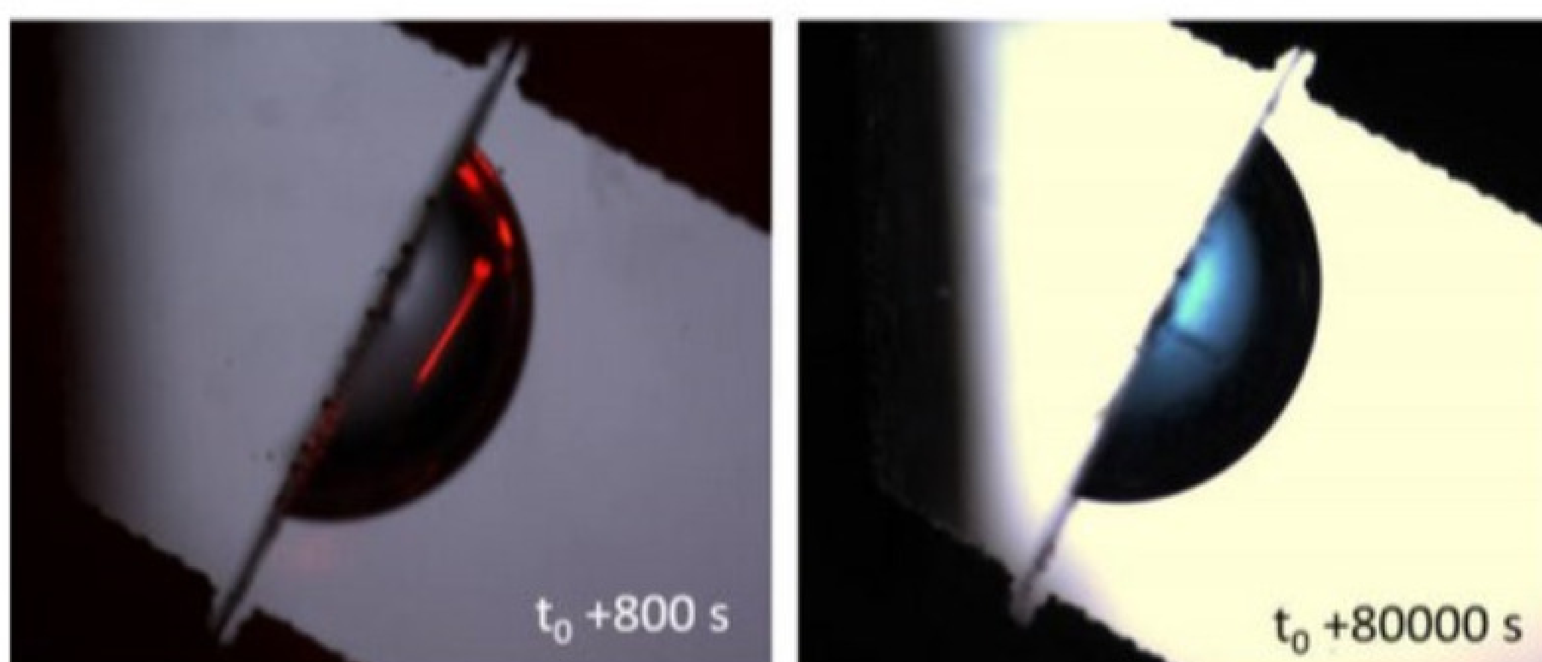


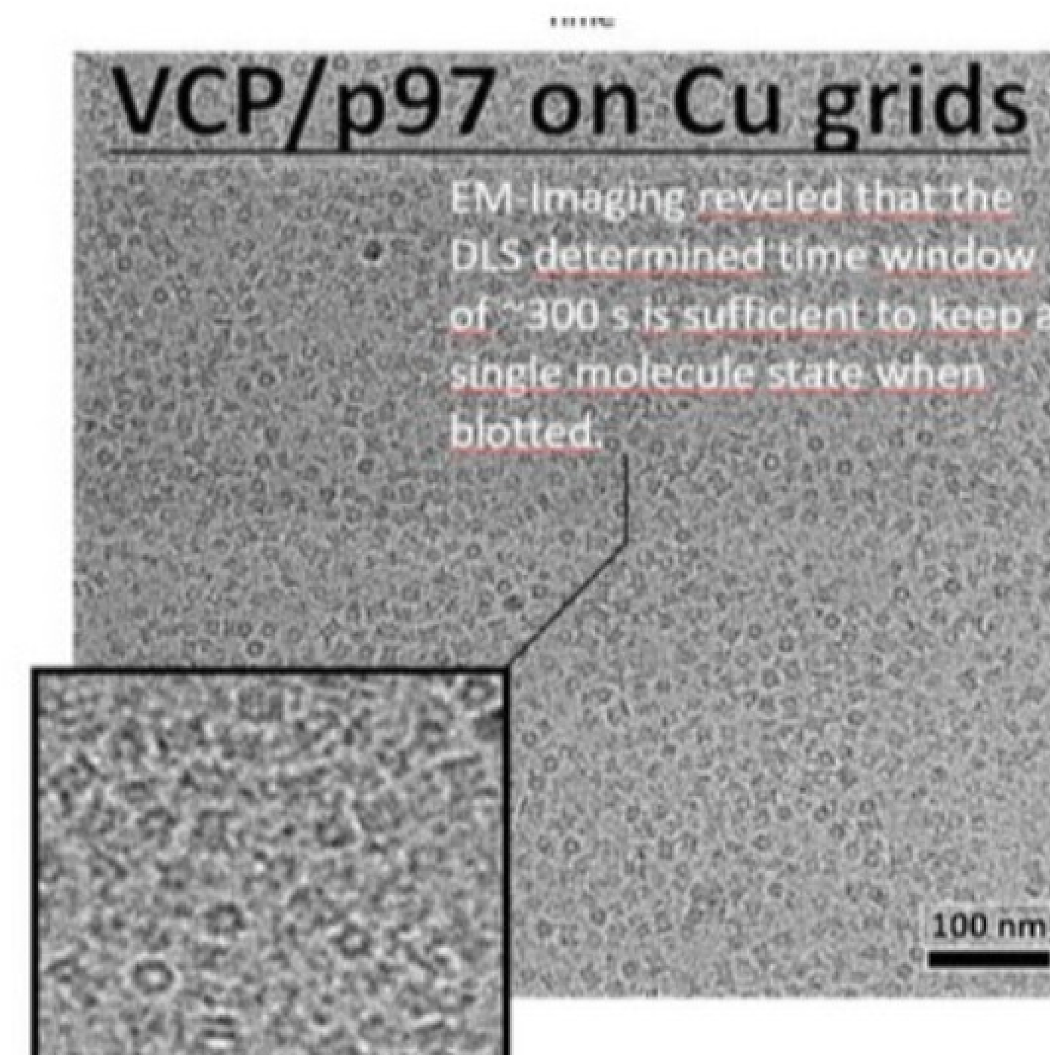
The Grid Material itself induces Changes in the Sample

As a sample carrier, the grid should not alter the sample in any way. However, due to the corrosive nature of the sample buffer, copper is dissolved as copper(II) ions and distributed uniformly throughout the sample volume. There's a threshold concentration of copper(II) ions that a protein can tolerate. As soon as this threshold is exceeded by continuous copper dissolution, the protein starts to aggregate everywhere at the same time and growth rate. It is protein and buffer dependent when this effect occurs.



After a long incubation, the sample drop will turn bluish, indicating the presence of copper(II) ions. However, the detected stability of 300 s on the grid was sufficient to maintain VPC/p97 particle uniformity and separation on the grid after blotting.





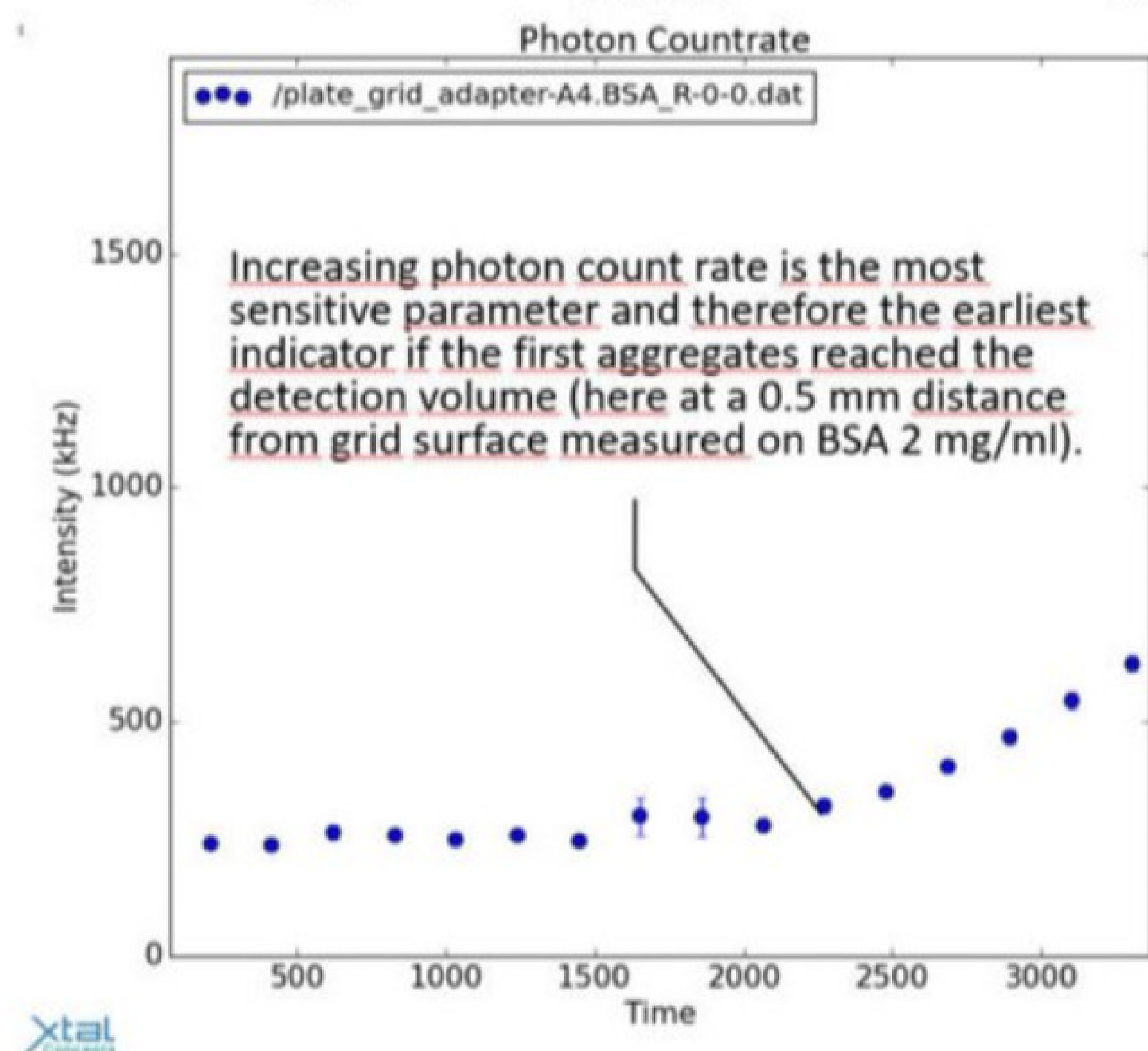
How relevant is it?

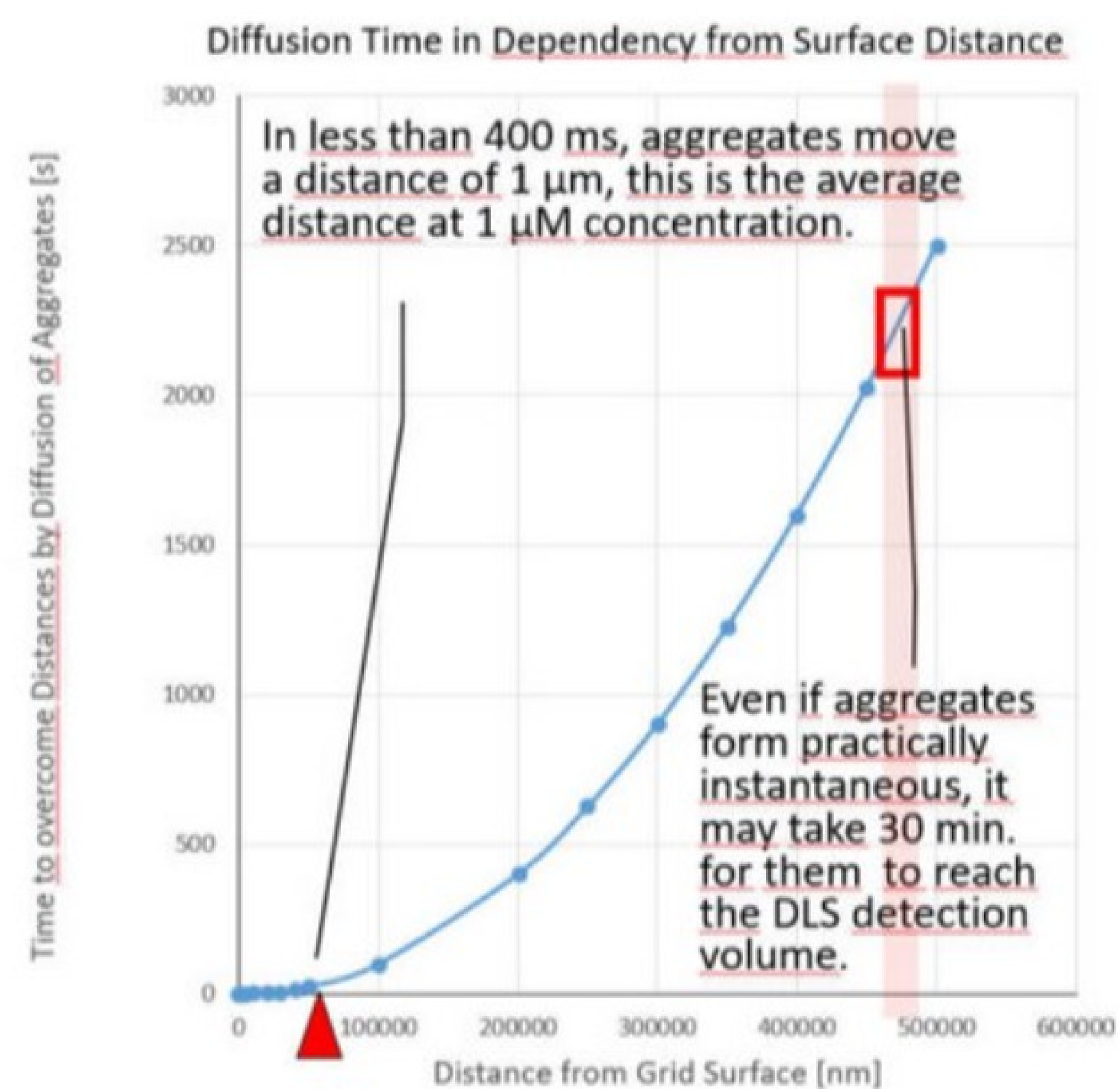
The most frequent comment about "on grid"-DLS is: "Although aggregation can be detected after 300 s, blotting is a matter of seconds. So what?" And the answer is: If a threshold concentration size change signature is detected, this is already a warning sign. If it happens after 300 s or later, it might be OK, but if it happens earlier, it is not.

Delayed DLS Detection of Aggregates even when they have formed almost instantaneously

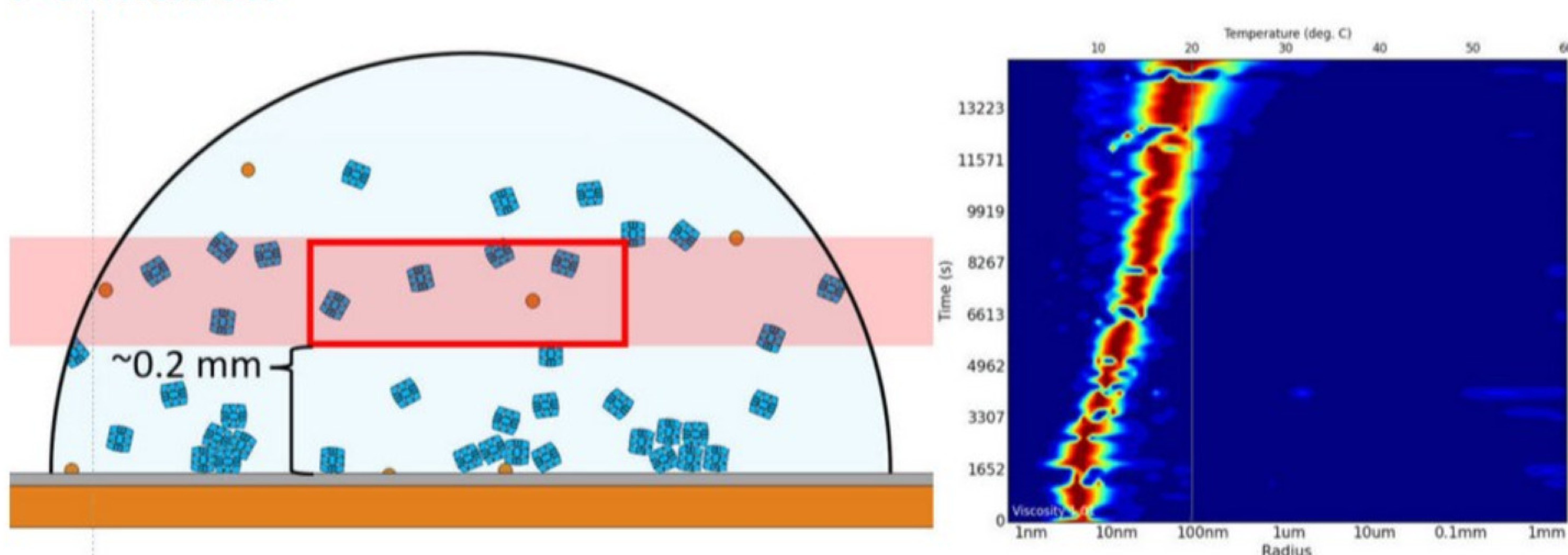
Aggregates are always detected with a delay. Even if they have formed almost instantaneously on the surface of the grid, they have to diffuse into the DLS detection volume over a distance of about 0.2 to 0.5 mm. This can take 15 minutes or more causing the delay. The formation of aggregates, on the other hand, is quite rapid. The average distance between molecules (which is often less than a few hundred nm) for a given protein diffusion coefficient (D) is overcome in the μs range and hence much faster than the blotting procedure itself.

$$\mathrm{D} = \frac{m^2}{s} = \frac{0.00000025\ m^2}{2500\ s} \sim 10^{-10}\ \frac{m^2}{s}$$



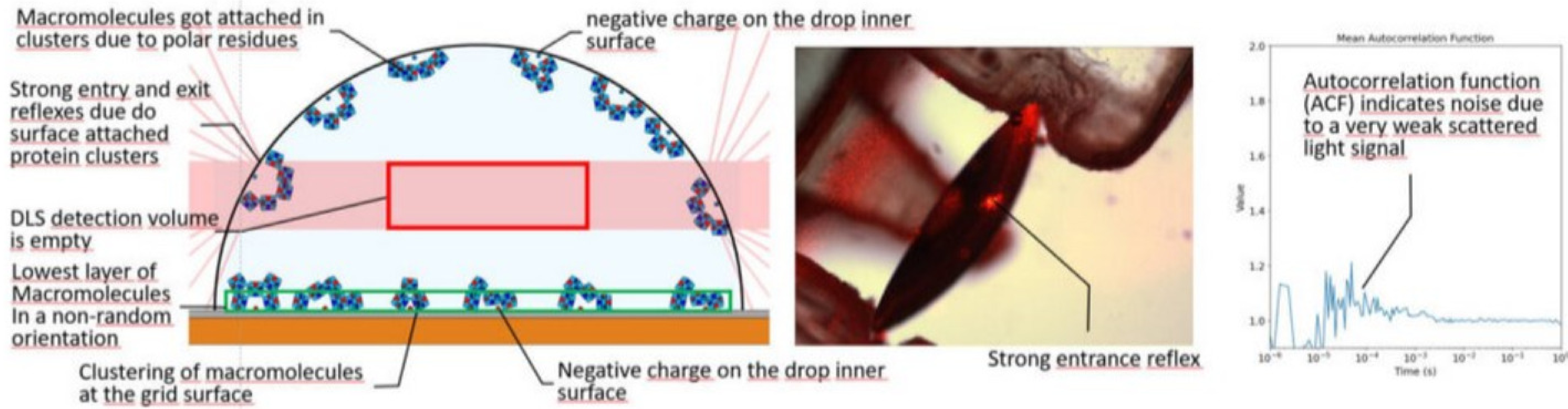


Delayed Aggregate Detection after instant Formation

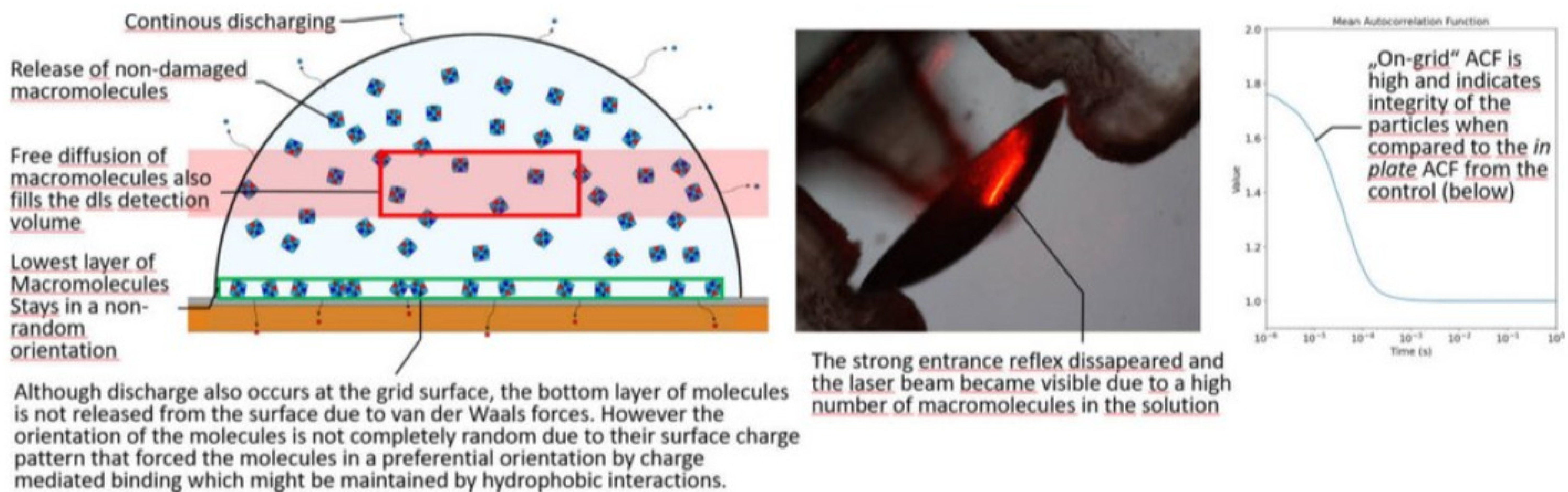


Preferential Orientation is supported by Surface Charge

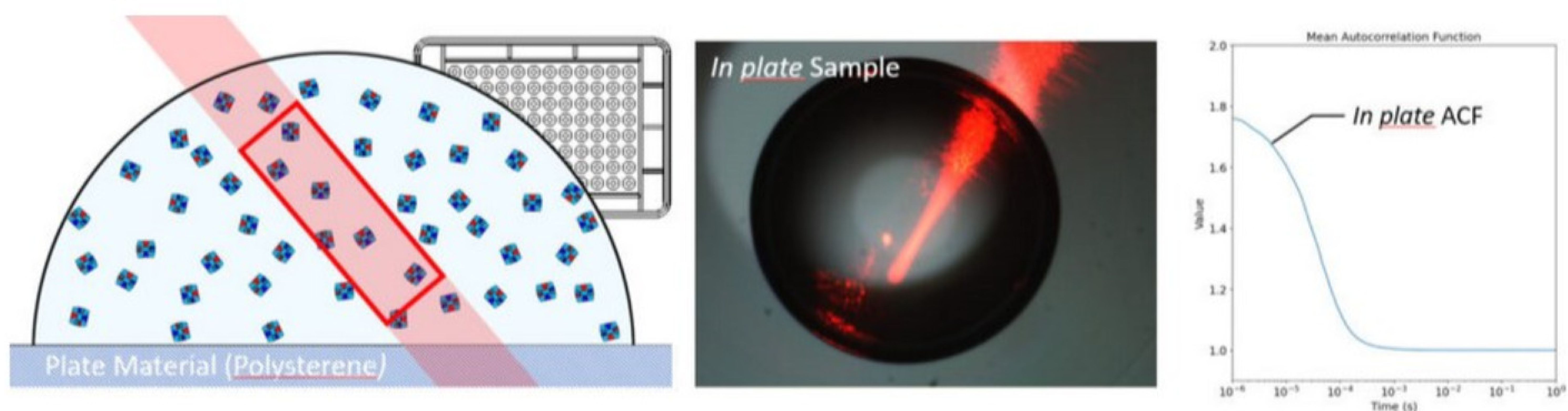
Preferential orientation could be supported by surface charge, as shown by the example of apoferritin on a copper grid, previously treated by glow discharge. At t0 +0 s no DLS laser beam was visible, only strong entrance and exit reflexes. The intercept of the autocorrelation function was very low and the signal noisy due to less scattered light from the sample.



At t0 +7200 s continuous Discharge leads to a release of the former attached Macromolecules



In plate Control Experiment at t0 +7200 s confirms Sample Integrity



Can the Charge induced preferential Orientation Problem be solved?

Probably yes. Discharging would eventually lead to a hydrophobic surface. However, it appears that a grid can remain charged and therefore hydrophilic for quite some time, so there's probably a value for how long a grid can be incubated before sample loading without losing its hydrophilic surface property. The key is to saturate the remaining surface charges with some protein molecules, but still have enough protein molecules in free diffusion to bind to the surface in random orientations during the freezing process.

The ACF is the key indicator for this, as it is caused by free translation diffusion, but this also means free rotational diffusion. As a long wetting of the grid surface is observed by simultaneous DLS/ACF detection, free rotational diffusion is taking place in the sample. The remaining surface charges, which provide the hydrophilic properties of the grid surface, are saturated with some of the protein. However, some protein remains in solution to bind in random orientations after blotting.

Once the value for the grid incubation time has been identified, it can always be used for this particular sample.

Acknowledgements

We would like to thank Stephan Übel, Daniel Bolschweiler and Florian Brod from the Max Planck Institute of Biochemistry Am Klopferspitz 18 82152 Martinsried, Germany for their kind advice and support. This work would not have been possible without their contribution.

Dynamic Light Scattering on Cryo-EM grids were performed by SpectroLight 600

DLS on cryo-EM grids can be performed using the same hardware "SpectroLight 600", a table top plate scanning DLS instrument with some unique features, such as its unrivalled small sample volume (80-800 nl/well) and its outstanding versatility despite being a fully automated scanning DLS system.

[Please visit our website to find out more about the SpectroLight 600.]

SpectroLight 600

Your XtalConcepts Team

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