\*\*NAGpkin\*\* is a free web tool for characterizing the mechanisms of protein phase separation. Protein phase separation is relevant in both health and disease in phenomena such as the liquid-liquid phase separation of functional droplets, or the self-assembly of proteins into solid aggregates called amyloids.

- What do I need?

You just need to upload into section \*\*ANALYSE\*\* the raw data describing the formation of a new protein phase. You can choose between mass-based or size-based progress curves.

- What are mass-based progress curves?

In mass-based progress curves the mass of the new phase is given as a function of time. Indirect measurements such as thioflavin-T fluorescence are also possible.

- What are size-based progress curves?

Size-based progress curves give information about the evolution of the mean size with time. This size can be measured using, for example, image analysis tools or dynamic light scattering.

- What information is provided?

\*\*NAGpkin\*\* quantifies the relative importance of the kinetic steps of primary nucleation, secondary nucleation and growth. Information about the possible occurrence of [Off-Pathway Aggregation](https://doi.org/10.3390/biom8040108), [Surface Tension Effects](https://doi.org/10.1101/2022.11.23.517626) and coalescence is also provided.

- How good are NAGpkin predictions?

For better results, progress curves measured at different protein concentrations [P] should be used as input to \*\*NAGpkin\*\*. Since only two or three kinetic parameters are fitted to mass- or size-based progress curves, respectively, the use of different [P] values decreases the degrees of freedom (and uncertainty) associated with \*\*NAGpkin\*\* predictions. In addition, r-squared values are provided to quantify the goodness of fit.

- How can NAGpkin predictions be tested?

You can test \*NAGpkin\*\* predictions by measuring the size distribution of the new phase. This will provide you with important parameters such as the mean size, variance, or the shape of the distribution at a given point of the phase separation. Then, you can compare the measured distribution with the one predicted in section \*\*PREDICT\*\* using the parameters previously fitted in section \*\*ANALYSE\*\*.

-Where can I find more information?

A report summarizing the main conclusions of the analysis is provided in section \*\*ANALYSE\*\* together with the links to bibliographic references.

- [Here](), you can find the Crystallization-Like Model (CLM) describing mass-based progress curves with only two kinetic parameters. These parameters characterize primary nucleation and the combined influence of the autocatalytic processes of secondary nucleation and growth.

- Here](), you can know how to identify off-pathway aggregation from mass-based progress curves.

- Here](), you can find the CLM extended to consider size-based progress curves.

- Here(), you can find the CLM extended to liquid-liquid phase separation processes, and how to predict particle size distribution from the fitted kinetic parameters.

- More application examples of the CLM can be found [here](https://doi.org/10.1021/acs.jpcb.7b01120) (on the effect of molecular crowding), [here]( https://doi.org/10.1002/asia.201801703) (on applications to drug discovery), and [here]( https://doi.org/10.3390/biom8040108) (on the use of kinetic scaling laws).