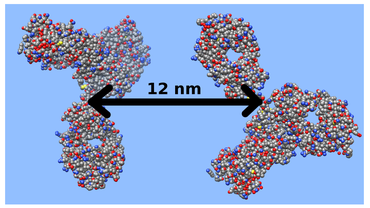
Colloidal Interactions and Protein Aggregation In-silico

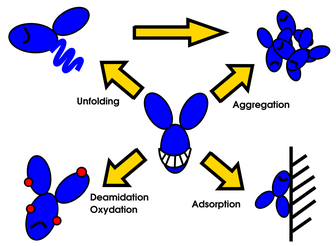
Michael Brunsteiner and Bernd Nidetzky

Research Center Pharmaceutical Engineering and Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Graz, Austria

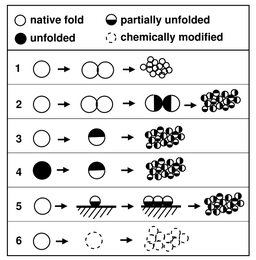
THE PROBLEM



State of the art formulations of bio-pharmaceuticals have protein concentrations $\geq$100 g/l.



Numerous processes can reduce the shelf-live of concentrated protein solutions.

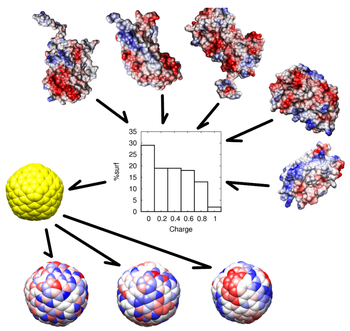


Aggregation alone can proceed through several mechanisms or combinations thereof.

- pH, ionic strength, buffer-type, concentration and type of osmolytes and surfactants, etc need to be optimized during the development of stable protein formulations.
- Effects of these parameters are complex and inter-dependent: development of formulations is a multi-dimensional optimization problem requiring a large number of expensive and time-consuming experiments.
- A theoretical prediction of aggregation propensities based on simple physico-chemical descriptors would reduce the required experimental effort and/or allow a more targeted exploration pf parameter space.
- Algorithms, based on, e.g., net-charge and hydrophobicity (SAP)[1] have been proposed, but not been tested extensively, due to a lack of comparable experimental data in the public domain.

THE APPROACH

- We developed a simple protein model designed to scrutinize some of the assumptions that existing algorithms for the prediction of aggregation propensities are based upon.
- Molecular Dynamics (MD) simulations were performed to calculate the free energy of association, ΔG(dist) (potential of mean force) between two proteins, and determine the relative influence of three descriptors: net-charge, dipole-moment, and hydrophobicity on colloidal interactions between proteins in aqueous solution.



Hydrophobicity (size of hydrophobic patches):

$$\mathrm{QH}_i = \left(q_i^2 + \sum_{j,r_{ij}<d_{\max}} q_j^2 / r_{ij}^5\right)^{-1}$$

Surface charge variation (homogeneity of surface charge distribution):

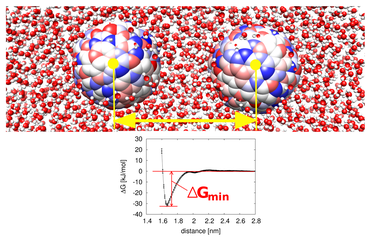
$$\mathrm{SCV} = N\left(\sum_i \left[\sum_{j,r_{ij}<d_{\max}} |q_i - q_j|\right]\right)^{-1}$$

Net-charge:

$$\mathrm{q} = \sum_i q_i$$

Dipole moment:

$$\mathrm{p} = \left(\sum_i q_i r_i\right) - \left(\sum_i q_i\right) r_{COM}$$

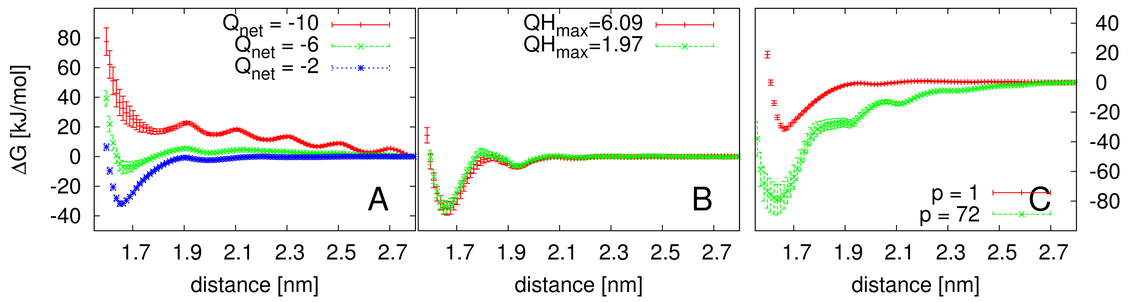


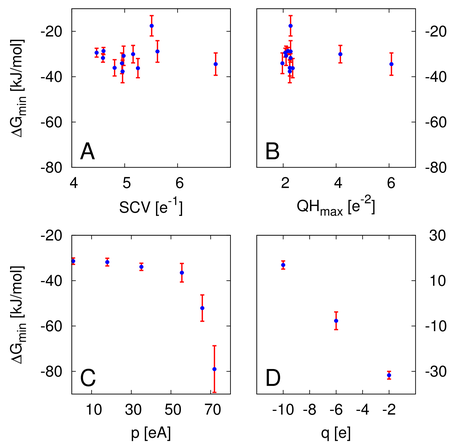
The model: Establish the average charge distribution on protein surfaces, assign charges to the atoms of a C_{240} fullerene $\longrightarrow$ pseudo-proteins, PP.

The descriptors: For a large number of PPs calculate the four descriptors and select instances covering a range of values for each descriptor.

The experiment: Perform MD simulations to calculate the first minimum of ΔG(dist) $\longrightarrow$ ΔG_{min}, free energy of association, a measure for colloidal interactions.

RESULTS





- Consider PPs with
  - q = -2 to -10 esu.
  - p = 1 to 72 eÅ.
  - QH_{max} = 2 to 6.1 esu^{-1} (corresponding to hydrophobic patches with approximately 2 – 5 Å radius)
- ΔG_{min} shows a strong variation and clear correlation with p and q.
- The variation of ΔG_{min} with p and q is comparable in size for p and q values typical for globular proteins.
- By comparison the variation with QH(hydrophobic patch-size) is weak, and there is no clear correlation with ΔG_{min}.

WHAT IT MEANS

- The accuracy of the presented results is un-precedented due to the usage of an explicit water model.
- A proteins dipole-moment and net-charge have effects of similar size on colloidal interactions. For most proteins a single mutation can reduce the dipole moment by 50-100%. In many cases the resulting variation of the attraction between two proteins is comparable to the effect of increasing the net-charge by up to ten elementary charge units. Including the dipole-moment as criterion for mutations is expected to require fewer mutations for the same stabilizing effect, reducing the risk of immunogenetic effects.
- Since hydrophobic patches seem to have a negligible impact on colloidal interactions we speculate that the removal of hydrophobic patches improves thermal, rather than colloidal stability.
- A simple and efficient statistical model for the prediction of aggregation propensities would include as descriptors net-charge and dipole moment, and an additional descriptor for thermal stability.

REFERENCES

[1] T. Lauer et al. (2012) Developability index: A rapid in silico tool for the screening of antibody aggregation propensity. *Journal of pharmaceutical sciences* 101(1):102-115

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Mail: michael.brunsteiner@tugraz.at WWW: http://www.rcpe.at