

Evaluation of mathematical and experimental approaches used to derive low-affinity equilibrium dissociation constants from electrospray binding fraction measurements

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Most of the cell functions that endow organisms with sustainable life rely on the interaction of a protein with ligands (proteins, nucleic acids, glycans, and/or small molecules). These interactions are often mediated by non-covalent bonds (hydrogen bonds, ionic, van der Waals, and hydrophobic interactions). To understand the biological role of a protein, it is important to obtain detailed information about these interactions, such as the equilibrium dissociation constant.

Electrospray ionization (ESI) mass spectrometry has gained interest as a rapid and sensitive tool to derive binding constants for non-covalent interactions. However, when using MS-based methods, the observation of the interaction is evidently made in the gas phase. Due to the optimization of conditions for desolvation and transmission in the mass spectrometer, solution phase equilibria can be significantly altered upon transfer to the gas phase.

In contrast to high-affinity complexes, where stoichiometry can be directly observed, low-affinity complexes in the gas-phase do not directly reflect the liquid phase equilibria. First, the interaction is so weak that avoiding partial dissociation of the specific complex in the atmospheric interface of the instrument becomes quite challenging. As a consequence, the bound fraction tends to be underestimated depending on the extent of gas-phase dissociation. Secondly, to observe the complex, it is necessary to employ a large excess of ligand, which leads to non-specific interactions called gaseous aggregation. The observed complex stoichiometry is then altered compared to the solution-phase stoichiometry.

At this time, two mathematical treatment methods have been developed to determine the binding constant of low-affinity complexes. The Daubenfeld method [1] is based on the hypothesis that the ligand binds both in a specific and a non-specific (aggregation) manner to the protein, and that the binding modes follow two different statistical distributions. By deconvoluting the raw fraction data into separate distributions, the authors obtain the part of the interaction corresponding to specific binding. The binding constant determined this way is unimpaired by aggregation. The limits of this method are that a) it can't be applied "as is" to

cooperative binding and that b) it only deals with the aggregation problem and does not correct for gas-phase dissociation. The Czuczy method [2] takes into account the partial dissociation of the complex in the interface of the instrument and corrects the bound fraction to determine the dissociation constant. Disadvantages of this second method are that it doesn't provide a completely rational basis for the correction, and that it doesn't solve the aggregation problem. A third, experimental-side method, is based on physically removing the excess ligand to prevent gas-phase aggregation. This, in principle, allows for a more accurate stoichiometry assessment. The limits of this method are that a) it does not address the dissociation issue and b) it is not explicitly designed for low-affinity systems. As a consequence, it does not take into account the fact that a low-affinity system with a high kinetic dissociation constant k_{off} is likely to partially dissociate through mass action law after removal of the free ligand.

In practice, some of these issues may cancel each other out at least in part, so that the determined K_D value is within an order of magnitude of the solution-phase K_D , or less. However, there is ample room for improvement towards making MS measurement of non-covalent complexes as direct and reliable as solution-phase measurements, with the speed and molecular accuracy afforded by MS methods.

Taking into account the need to correct for the aggregation on one hand and for the partial dissociation in the interface on the other hand, we have developed a combined mathematical and experimental approach. After incubation of the partners in solution, the excess ligand is eliminated using micro-desalting methods immediately prior to ESI-MS analysis. Then, a mathematical treatment is applied to the fractional binding MS data to account for gas-phase dissociation as well as dissociation resulting from the micro-desalting in low-affinity systems. Our model and its ability to fit experimental data will be presented. A comparison of our K_D results with values determined in solution will provide validation for our approach.

[1] Daubenfeld T, Bouin A, Van der Rest G. 2006. *JASMS* 17(9):1239-1248. [2] Czuczy N, Takáts Z. *Poster comm. at the 18th IMSC 2009*.