



# Cracking Complexes To Build Models of Protein Assemblies

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**Surface-induced dissociation mass spectrometry as a method to better predict protein complex structure.**

Cellular proteins are social molecules. They form vast interaction networks, where proteins assemble together with other partners<sup>1</sup> to perform a multitude of cellular functions, from making new proteins (ribosomes) to creating the chemical energy needed to power the cell (ATP synthases) and even degrading proteins (proteasomes) so that new ones can be made. Protein–protein interactions are also key mediators of cell signaling and recognition.

Understanding the chemical detail of these binding interactions not only gives us a deeper view of the cellular interactome, and how protein association has evolved<sup>2</sup> but also is crucial for creating designer interfaces with desired binding properties.<sup>3</sup> Computational approaches, such as macromolecular docking, allow many orientations of binding partners to be screened in silico, to predict interfaces between protein subunits<sup>4</sup> and the architecture of polydisperse assemblies. Complementing this strategy with even minimal experimental data can vastly speed up this heavy computational task and increase the accuracy of models. Typically, docking approaches involve measuring and integrating properties of the intact assembly such as distance restraints between residues on different binding partners. However, in this issue of *ACS Central Science*,<sup>5</sup> the Wysocki and Lindert research groups have joined forces to tackle this problem almost in reverse. By splitting protein assemblies apart, they propose new models to predict how proteins bind together.

By combining native mass spectrometry (nMS) with an extremely fast activation technique—surface-induced dissociation (SID)—they first preserve then break apart these complexes. nMS involves gently ionizing proteins and noncovalent protein assemblies, and transferring them from solution into the gas phase while maintaining features

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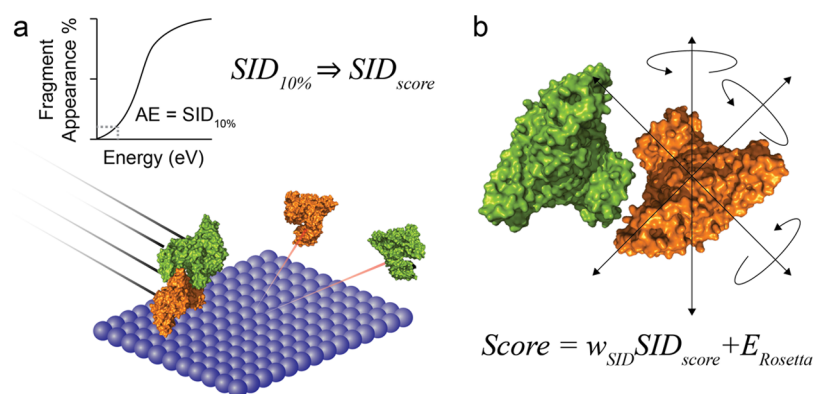
of their solution structure, such as noncovalent interactions and overall fold.<sup>6</sup> Once inside the mass spectrometer, these carefully preserved assemblies can be disrupted by collisional activation. Typically, this is achieved by accelerating ions into a high-pressure collision cell, where many repeated collisions with buffer gas molecules slowly heat and unfold the protein assembly. Eventually, one or more subunits are unfolded and ejected. Here the authors use SID to deposit energy much more quickly than by collisional activation, thereby shattering complexes into folded subunits rather than ejecting unfolded monomers.<sup>7</sup>

SID AE data provide useful restraints for in-solution modeling.

Exploiting this unique property of SID, Wysocki and Lindert have recently shown that the experimentally determined SID appearance energy (AE), defined as 10% fragment appearance, can be correlated with features of the protein–protein interface, such as the number of unsatisfied hydrogen bonds, interacting residues, and subunit rigidity.<sup>8</sup> In this paper, they show that a modified set of interface properties, this time incorporating the buried hydrophobic interface surface area together with interacting residues and subunit rigidity, can be used to successfully predict SID AE from docking poses of interacting proteins generated in silico. With this predictive power in hand, they go on to

**Published:** August 13, 2019





**Figure 1.** Integrating SID data into macromolecular docking helps score and select more realistic protein poses. (a) Complexes are dissociated by collision with a solid surface inside the mass spectrometer. Monitoring the extent of fragment appearance (%) allows measurement of the SID appearance energy (AE) which incorporates interface properties such as number of interacting residues, buried surface area, and subunit rigidity. (b) Models of interacting proteins are generated *in silico* and ranked based on a likelihood score integrating a comparison of experimental and predicted AE and combined with RosettaDock.

create a scoring function (SID score) incorporating the SID AE, which they have integrated into the popular modeling environment known as Rosetta.

After successfully testing the SID AE scoring function on a set of 57 known structures where AE was generated using the predictive model, they then use the experimentally determined SID AE's of nine protein complexes to rescore poses generated *in silico*. They show that their new approach incorporating the SID score outperforms the standard Rosetta scoring function in selecting poses that correlate more closely with crystal structures. Importantly, they propose that SID AE data provide useful restraints for *in-solution* modeling.

More generally, as the understanding of the fundamental mechanisms behind SID evolves, further beneficiaries of the information SID can provide, about the nature of protein–protein interfaces, will emerge. It is also noteworthy that such biophysical data, obtained in the gas phase, can be correlated to biologically relevant solution phase structural information. This not only represents both an important advance for this powerful fragmentation technique but also highlights a growing trend of using nMS to improve, or create new, characterization workflows. For example, automated intact mass measurement of noncovalent assemblies and biotherapeutics is now being employed by a number of researchers.<sup>9</sup> nMS is also being trialled for high-throughput drug/ligand screening in industry,<sup>10</sup> and its ability to resolve multiple protein complexes is being deployed to guide conditions for high-resolution cryo-EM.<sup>11,12</sup> This proliferation of nMS, and the associated rapid expansion of the user base, will undoubtedly result in other powerful and exciting combinations. Together with advanced computation, we anticipate that these nMS

approaches will provide a disruptive impact that will deepen our understanding of the biological world.

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#### Notes

The authors declare no competing financial interest.

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