I. Intact mass determination – stoichiometry

Here we show two examples to demonstrate the utility of native MS in determining the number of components in a protein complex. One example is a Nanodisc lipoprotein complex (Fig. 1). Nanodiscs, which are nanoscale lipid bilayers encircled and held intact by engineered membrane scaffold proteins (MSP), can solubilize membrane proteins for analysis. The scaffold protein defines the diameter of the nanodisc complex while the type of lipid defines how many lipids are required to pack the bilayer area inside the MSP perimeter (Fig. 1A). Given that nanodiscs contain a native-like lipid bilayer, they provide a more physiologically relevant environment to study membrane protein complexes than do micelles. Nanodiscs are also more monodisperse than detergent micelles, and the lipid content of the nanodisc can be tailored to permit investigation of protein−lipid interactions.

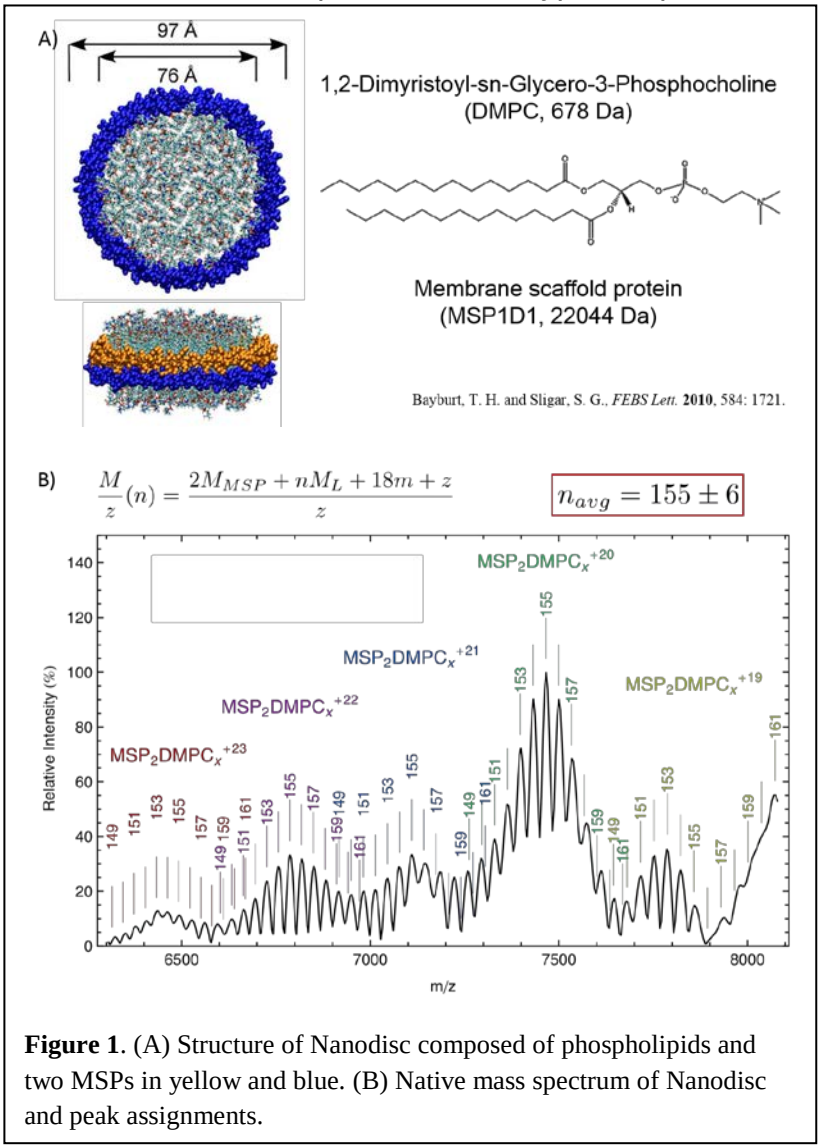

Figure 1. (A) Structure of Nanodisc composed of phospholipids and two MSPs in yellow and blue. (B) Native mass spectrum of Nanodisc and peak assignments.

Nanodiscs were used previously in several MS applications, but in those studies, the researchers disassembled the nanodiscs prior to analysis. We showed the first evidence that intact nanodisc complexes can be introduced to the gas phase of a mass spectrometer using native electrospray MS for direct measurement of their molecular weight and size dispersity (Fig. 1B).

The native mass spectra for DMPC nanodiscs as measured by FTICR mass spectrometry show a fine spacing within each broad peak (Figures 1B). We hypothesize that this spacing is due to slight differences in lipid packing, with each peak representing a nanodisc with a defined number of lipids. Adjacent narrow peaks differ by the mass of a single lipid. Distributions in aggregation number were previously observed in native MS of detergent micelles[8,9] but this is the first direct observation of the lipid distribution (polydispersity) of nanodiscs. Accepting the above

hypothesis, we determined the charge for each species by dividing the mass of the lipid by the spacing between adjacent peaks. The three broad peaks, corresponding to the +22, +21, and +20 charge states, were fit to three overlapping Gaussian distributions to calculate the average MW and polydispersity. The mean and standard deviations of the three distributions were averaged, and errors were reported as 1 standard deviation. Fitting FTICR mass spectra to Gaussian distributions shows that nanodiscs containing DMPC have an average mass of 149.5 ± 0.5 kDa and are sprayed with an average charge state of +21 at 70 V CAD. These DMPC nanodiscs average 155 lipids per nanodisc or 77.5 lipids per leaflet. This lipid total agrees well with the 77 lipids per leaflet, previously measured for DMPC by radioactive methods. The average lipid count has a standard deviation of 2.4 ± 0.5 lipids per leaflet. These measurements provide important fundamental characterization of nanodiscs and will serve as a basis for future studies using nanodiscs as a platform for studying membrane proteins using native MS.

Another example is photosynthetic antenna protein from green sulfur bacteria called FMO (Fenna-Mathews-Olson) protein. Three copies of this protein and a number of pigments/co-factors form a functional unit to direct energy from the chlorosome to the reaction center (Figs. 2A, 2B and 2C). The pigment inside FMO is bacteriochlorophyll (BChl) a, a highly colored pigment that contains a porphyrin-like ring structure (Fig. 2D). Each FMO subunit is formed mainly by β sheets, like a compact ‘‘taco shell’’ containing α helices and loops on the open end, which encloses a central core of seven BChl a pigments. High-resolution structures of FMO protein suggested the presence of an eighth pigment BChl a located in the connection region between the open end of ‘‘taco shell’’ FMO and the antenna chlorosomes. We applied native MS to examine the pigment stoichiometry problem by measuring MW. After buffer

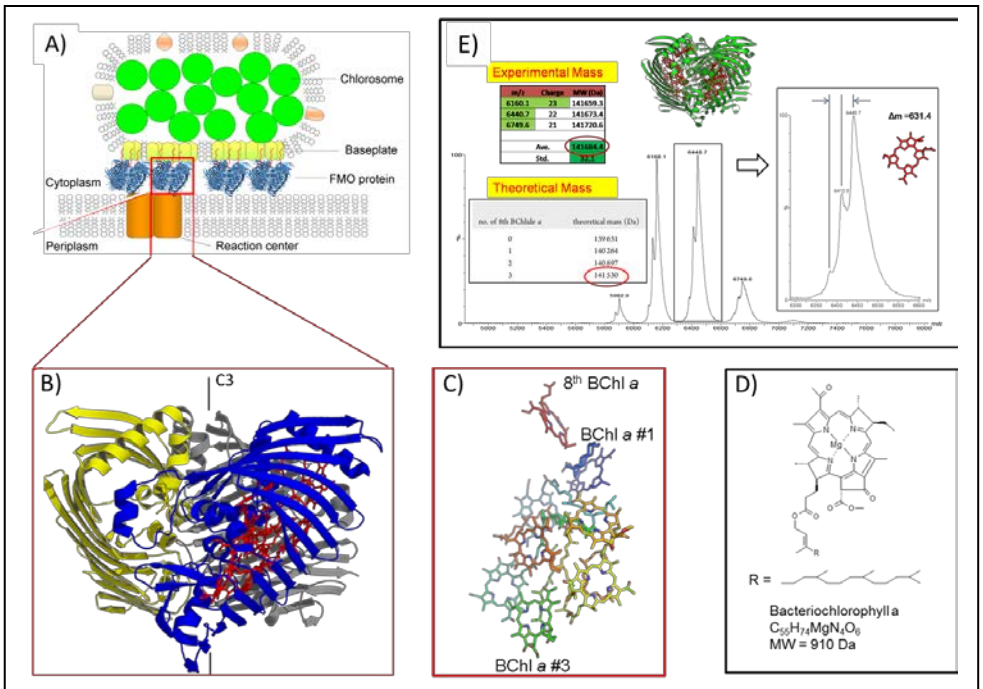

Figure 2. The photosynthetic system from green sulfur bacteria. (A) The components of the green sulfur bacterial photosystem; (B) X-ray crystal model of FMO protein complex. The three subunits are colored blue, yellow and gray. The BChl a pigments are colored red; (C) BChl a pigments of one subunit; (D) BChl a molecular structure; (E) Native MS of intact FMO protein complex.

exchange with ammonium acetate the purified intact FMO antenna protein complexes from Chlorobaculum tepidum (TFMO) and Prosthecochloris aestuarii (AFMO) were analyzed using native ESI of the intact complexes. We observed three to four charge states at high m/z range (Figs. 2C and 2E), allowing the MW of intact FMO complexes to be determined. The MW of FMO from native MS is ~141 kDa, which is greater than the total MW of three polypeptide chain subunits (39.8 kDa each). Clearly, the intact FMO complex containing three subunits can be preserved in the gas phase. We desolvated the intact FMO complex ions in the gas phase by applying CID to yield multiple species in each charge state of intact FMO complex (Fig. 2E). The mass differences between neighboring peaks are ~630 Da, which is less than the mass of the full *BChl a* pigment (910 Da), but close to the mass of bacteriochlorophyllide (632 Da), which lacks the isoprenoid phytyl tail found in the intact pigment. In fact, isolated BChl a readily fragments in a mass spectrometer by losing its phytyl chain. Thus, native MS provided the first experimental evidence of the existence of this eighth *BChl a* pigment in stoichiometric quantities. More details can be found in the references below.

References

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