1 Measurement Modalities and Literature Review

1.1 Instrumentation

The preceding sections enumerating the possible additive and subtractive transformations that can occur on amino acid polymers are the set-up for the main event: the sensitive single-molecule measurement. There is tremendous diversity in single-molecule methods, and several promising candidates will be described here.

1.1.1 Optical microscopy

If the reporter can be excited in order to emit photons (i.e. it fluoresces), it can be detected with a light microscope if it is sufficiently bright. The commonplace epifluorescence microscope, however, excites the whole sample volume, and the resulting background often makes single-molecule measurements impossible. We thus turn to two specialized types of light microscopes: the confocal microscope and the TIRF microscope. In confocal microscopy, contrast is enhanced by blocking out of focus light with a pinhole [1], and this is widely used for single-molecule protein measurements[2], although sequencing remains aspirational. In total internal reflection (TIRF) microscopy, the excitation light is fully reflected at glass-sample interface to produce an exponetially decaying evanescent wave that only excites fluorophores within approximately 100 nm of the surface [3]. This is also effective at enhancing contrast, again yielding single-molecule protein measurements as well as a proposal for single-molecule sequencing[4].

1.1.2 Nanopore

Here, a molecular machine is used to unfold and ratchet a protein through a pore; current across the pore is continuously measured and current modulations due to amino acid reporters can yield the acid's identity. This is one of the more experimentally substantiated approaches to proten sequencing, with a recent demonstration of protein translocation and epitope determination[5]. It should be noted that the reporter need not only modulate electrical measurements; a nanopore can be simultaneously monitored optically to detect e.g. cooperative interactions between fluorescent reporter dyes on the protein and on the nanopore [6].

1.1.3 Electron microscopy

Electron microscopes can measure electron density, and sensitive electron microscopes can discern differences in electron density between amino acids; these differences can also be accentuated by stains or other reporters. Impressive progress has been made recently determining 3D protein structure using cryo-electron microscopy, however, due to sample processing requirements it has yet to be proposed as serious or scalable method of protein sequencing [7].

1.1.4 Mass spectrometry

Mass spectrometry is the workhorse of current bulk protein sequencing efforts, and efforts are being made to extend its capabilities into the single molecule regime. Here, either a whole or fragmented protein is ionized and introduced into a mass analyser (e.g. quadrupole, time-of-flight). The resulting mass/charge ratio can fingerprint the fragment and bioinformatics approaches can verify its sequence [8]. Typically femtomole quantities of the protein or fragment is needed to generate a detectable signal; however, a recent landmark study permitted a single-molecule mass spectrum measurement of antibodies by tracking vibrational modes of a nanoelectromechanical resonator[9].

1.1.5 Force spectroscopy

Force-induced unfolding of proteins by optical tweezers or atomic force microscopy are well characterized single molecule methods which can generate force spectra which readily identify particular folding states and domains [10]. However, extending the analysis to reveal sequence information is challenging, and the highly technical nature of these experiments will make widespread adoption difficult.

1.1.6 NMR spectroscopy

Nuclear magnetic resonance spectroscopy can identify chemical information in molecules based on absorption and emission of RF pulses by nuclei in a magnetic field. This has been an important tool for structural studies of e.g. protein conformations, as well as a routine analytic tool in chemical sythesis, medicine, etc [11]. However, extending NMR into the single molcule regime is daunting, although efforts involving nitrogen vacancy centers in nanodiamonds shows promise [12]. That said, there is no clear path from the state of the art to a practical means of extracting protein sequence information.

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