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New methodologies for measuring protein interactions *in vivo* and *in vitro*

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The identification and characterization of protein interactions is a key topic in current life science research; a huge variety of methodologies have been established in recent years to expedite research in this area. Generic methods have been established for monitoring protein interactions *in vivo* by protein fragment complementation and for screening protein interactions *in vitro* by highly parallel solid-phase techniques. Substantial progress has been made in identifying and characterizing interactions with and between membrane proteins. Studying protein interactions on the single-molecule level has become an important tool for understanding protein function *in vivo* and *in vitro*.

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Abbreviations

AFM	atomic force microscopy/microscope
BRET	bioluminescence resonance energy transfer
DBD	DNA-binding domain
FRET	fluorescence resonance energy transfer
GFP	green fluorescent protein
GPCR	G-protein-coupled receptor
PCA	protein fragment complementation assay
Rif	reflectance interference
SPR	surface plasmon resonance
TAD	transcription activation domain
TAP	tandem affinity purification
TIRF	total internal reflection fluorescence
Y2H	yeast two-hybrid
YFP	yellow fluorescent protein

Introduction

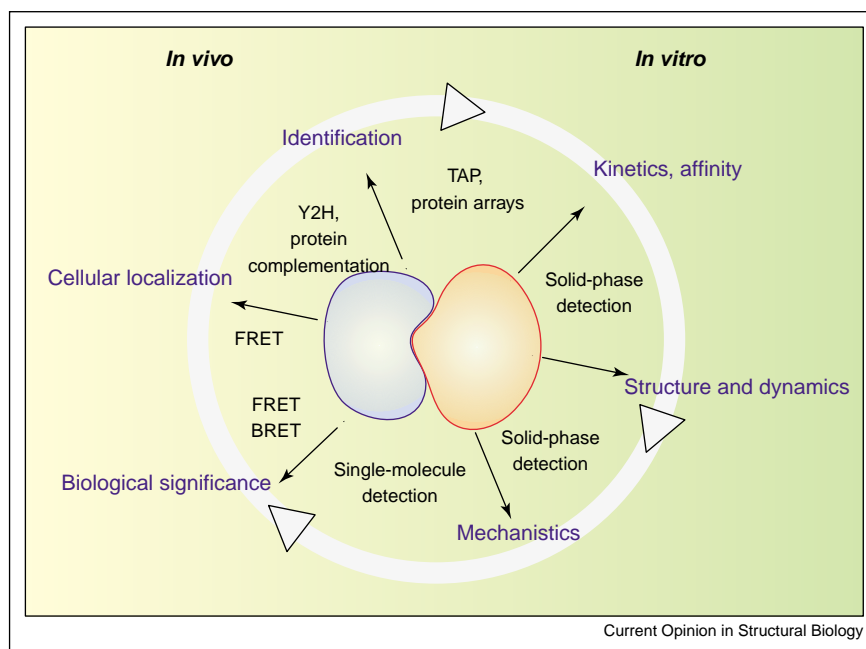
Faced with a constantly increasing number of sequenced genomes, life science research is now focusing on the properties and functions of the encoded proteins. One basic property of proteins is their ability to specifically target and form non-covalent complexes with other

proteins. Such protein–protein interactions play key roles in all cellular processes and functions. Identifying and characterizing protein interactions and entire interaction networks ('interactomes') is therefore prerequisite to understanding these processes on a molecular and biophysical level. An average of five interaction partners per protein has been estimated, predicting a problem that far exceeds the complexity of the genome. Furthermore, dissection of the interactome is highly challenging because of the physicochemically diverse properties of proteins and the very different characteristics of protein–protein interactions: equilibrium dissociation constants of protein complexes vary over several orders of magnitude; proteins have very different abundances in the cell and in its compartments; and different conditions are required for purifying and handling proteins *in vitro*.

Protein interactions can be analyzed from many perspectives (Figure 1). In order to identify protein interaction partners, techniques are required that enable the possible screening of large numbers of proteins and that should preferably operate *in vivo* to maintain the cellular context. Once specific and relevant interactions have been identified, the molecular and biophysical properties of the complexes have to be characterized in more detail. Key parameters are the oligomeric state of the interaction partner and the stoichiometric ratio in the complex, the affinity of the interaction partners for each other, the kinetic rate constants and the nature of the interaction sites. For this part of protein interaction analysis, purified and well-characterized proteins are required, often in comparably high amounts and concentrations. However, a detailed understanding of protein interactions on the molecular level also has to take into account the cellular environment, requiring techniques for studying interactions *in vivo*.

Given the plethora of established techniques for measuring protein interactions, the scope of this review is to survey and categorize generic and frequently applied techniques for identifying and characterizing protein interactions. Particular focus will be on the main challenges in protein interaction analysis: monitoring and analyzing protein interactions in living cells; studying interactions of membrane proteins and membrane-associated proteins (which account for up to 30% of the proteome of an organism, but are notoriously difficult to study); generic methods suitable for high-throughput analysis of protein interactions; and spectroscopic techniques for studying protein interactions on the single-molecule level, which promise to bridge *in vivo* and *in vitro* approaches.

Figure 1



Different levels of characterization of protein interactions *in vivo* and *in vitro*.

Identification of protein–protein interactions *in vivo*

Two-hybrid techniques

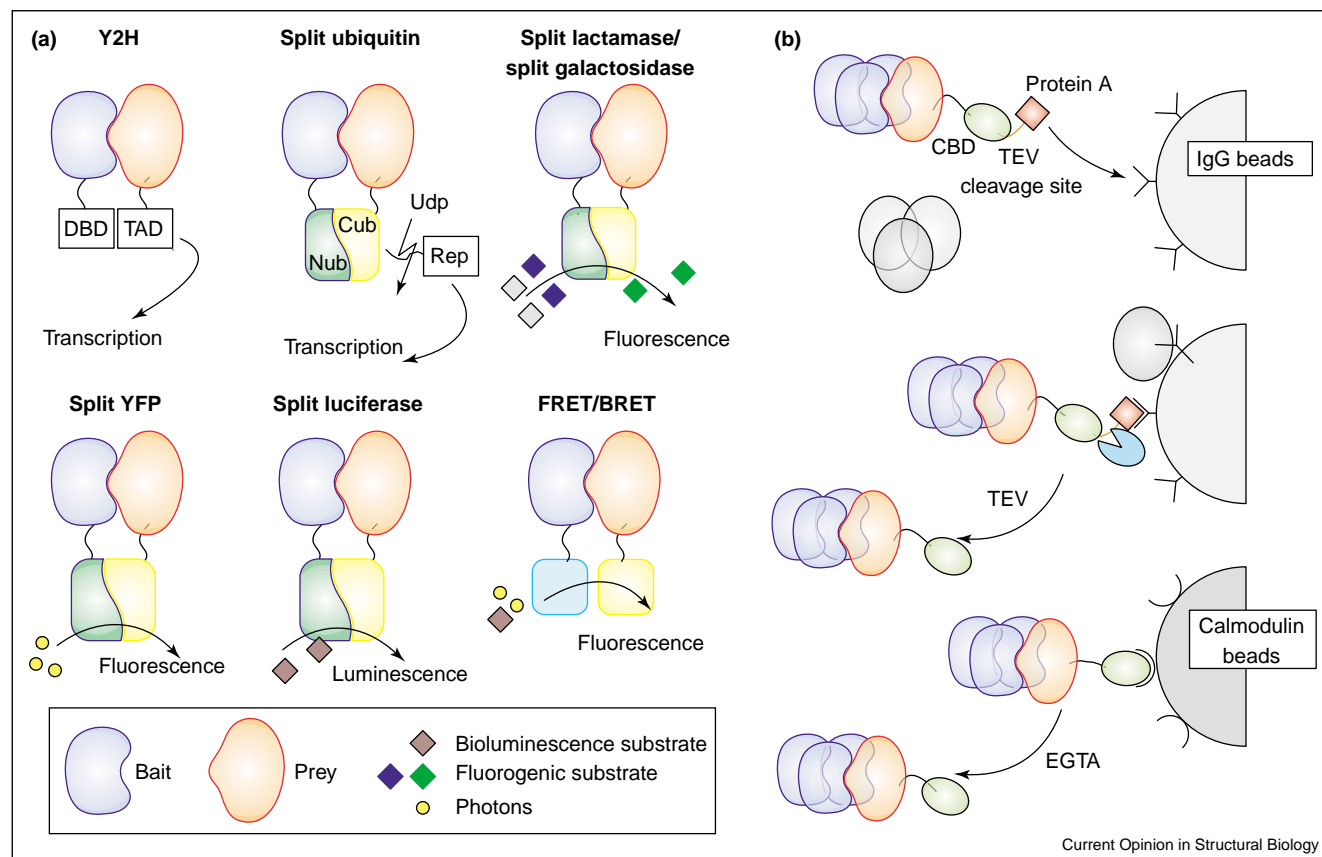
The screening and identification of protein–protein interactions within the cell requires suitable biological or spectroscopic reporter systems. A breakthrough in the screening of protein interactions *in vivo* was made 15 years ago by implementing the yeast two-hybrid (Y2H) system [1]. This technique was inspired by the modular structure of transcription factors containing a DNA-binding domain (DBD) and a transcription activation domain (TAD). Splitting DBD and TAD inactivated the transcription factor, but its function could be restored by fusing DBD and TAD to two interacting proteins. The Y2H technique utilizes this effect to identify protein interactions by fusing a ‘bait’ protein to a DBD and potential interaction partners (‘prey’ proteins) to the TAD (Figure 2a). The Y2H system today is a mature and very broadly applied method. Many improvements and variations of this technique have been reported, and have been extensively reviewed [2]. In recent years, this technique was used to screen the protein interaction networks and interactomes of yeast [3] and *Caenorhabditis elegans* [4]. Several successful two-hybrid systems for mammalian cells have also been reported [5], but so far these methods cannot compete with the popularity of the Y2H system.

Protein fragment complementation

The classic Y2H systems are limited to protein interactions in the nucleus; thus, interactions involving proteins

integrated into or anchored to the plasma membrane are barely accessible. This dilemma was resolved by extending the two-hybrid approach to protein fragment complementation assays (PCAs), which were first implemented in the split ubiquitin technique [6]. Ubiquitin was split into two inactive fragments (Nub and Cub), which were fused to the bait and the prey proteins, respectively. Upon interaction between bait and prey proteins, the function of ubiquitin is restored by complementation of its fragments, leading to the cleavage of a reporter protein by ubiquitin-dependent proteases (Figure 2a). Refined split ubiquitin techniques were particularly successfully applied to identifying interactions between membrane proteins using different reporter proteins [7–9] and have recently been employed in systematically screening membrane protein interactomes [10]. Other enzymes, such as adenylate cyclase [11] or an anthranilate isomerase [12], have been used for split-protein-based screening of protein interactions in different organisms. A common feature of these approaches, however, is the indirect and time-shifted response, as they rely on the transcription of the reporter gene, which is furthermore prone to generating high numbers of false positives. In order to provide techniques for monitoring protein interactions in living cells, several approaches based on PCA with a more direct read-out have been developed. The common principle of these techniques compared to classic two-hybrid techniques is that the reporter protein itself is split into two fragments, which are genetically fused to the potential interaction partners.

Figure 2



Identification of protein interactions *in vivo* and *in vitro*. **(a)** Y2H systems and PCAs, and their detection principles. Rep, reporter; Udp, ubiquitin-dependent protease. **(b)** TAP of protein complexes via the TAP tag using two sequential steps of purification (adapted from [47]). Cleavage by TEV protease in the first step and EGTA in the second ensures very high specificity and a minimum effect on the eluted protein complexes. CBD, calmodulin-binding domain.

Upon interaction of these proteins, the fragments complement each other and the function of the fragmented protein is restored. The reporter proteins are typically enzymes such as galactosidase [13], dihydrofolate reductase (DHFR) [14] or lactamase [15], which convert chromogenic or fluorogenic substrates (Figure 2a). PCAs have been successfully applied to identifying protein–protein interactions in different compartments of the cell and, importantly, also to monitoring interactions between membrane proteins [16,17]. The responses of these enzyme-based systems can be very fast, for example, a few minutes in case of the split lactamase system [15].

Enzyme-based techniques require incubation with the substrate, which needs to be optimized with respect to concentration and incubation time depending on the proteins of interest. Although enzymatic activity amplifies the signal, the substrate is a source of background signal and the product accumulates during the assay. For fast detection of interactions with low background signal, PCAs with direct spectroscopic read-outs have been

investigated. Recently, a PCA has been reported based on a split yellow fluorescent protein (YFP) [18]. Because the folding and the chromophore formation of YFP are rather slow, complex life-times of several minutes are required for successful complementation. Although this limits its application to tight binding complexes, it efficiently reduces the background. Other autofluorescent proteins, such as green fluorescent protein (GFP), have also been used in PCAs [19]. The possibility of spectroscopically discriminating different autofluorescent proteins has recently been exploited in multiplexed protein interaction assays on the single cell level [20••]. Using the fragments of four different autofluorescent proteins, seven spectrally distinguishable complexes were obtained by their combination that could be used for the simultaneous detection of different protein interactions. The application of protein complementation was recently further expanded by implementing assays based on split luciferases [21,22]. Whereas fluorescence-based approaches always suffer from cellular autofluorescence, bioluminescence can be measured in living cells with

extremely low background [23]. Based on this method, protein–protein interactions could be monitored even in living mice [22,24]. Another general approach related to PCA is based on protein splicing and has been applied to several reporter proteins, such as GFP [25] and firefly luciferase [26].

Y2H systems and PCAs are currently the most favorable techniques for dissecting interaction networks [4*,27]. These techniques, however, generate rather high numbers of false positive results. Such false positives are efficiently reduced by using the multiple complementary techniques now available [28].

Directly monitoring protein interactions *in vivo*: resonance energy transfer

Although Y2H and PCA are potent techniques for identifying interactions, the real-time monitoring and localization of protein interactions in living cells requires an instant spectroscopic read-out. The principal challenge for direct spectroscopic investigation of protein interactions *in vivo* is the requirement for specifically attaching spectroscopic probes to the protein of interest in the cellular environment. Since the discovery of autofluorescent proteins, their application to characterizing protein–protein interactions in living cells has led to stunning developments that have been reviewed extensively [29,30]. The spectroscopic and physicochemical properties of autofluorescent proteins are constantly being improved and novel species are being discovered. However, other versatile techniques for spectroscopic labeling *in vivo* have also been reported. A novel promising technique for highly specific covalent labeling involves fusing a genetically engineered alkylguanine-DNA alkyltransferase (AGT) to the protein of interest [31**]. The spectroscopic probe is subsequently attached to the active site of AGT using a suicide substrate to which the probe is coupled. This approach considerably increases the possibility of selectively attaching not only different synthetic fluorescent probes but also fluorescence quenchers or spin labels.

Currently the most powerful technique for the direct spectroscopic detection and monitoring of protein interactions in living cells is fluorescence resonance energy transfer (FRET). FRET is a radiation-less energy transition between a donor and an acceptor fluorophore that occurs with finite probability only if the fluorophores are less than 10 nm apart. Thus, FRET can be used as a probe for close proximity upon interaction [32]. FRET can be readily detected by the change in emission intensity of the donor and the acceptor, and also by a change in the fluorescence life-time [33]. FRET is possible between different variants of GFP [34–36]. The detection of protein interactions in living cells by FRET can be carried out conveniently and with high throughput by flow cytometry [37]. The powerful features of FRET in

living cells, however, are fully made use of by fluorescence microscopy techniques [38]. Recent developments in fluorescence microscopy have further boosted the versatility of FRET in living cells [39]. In particular, fluorescence life-time imaging [40] is a powerful microscopy technique for FRET imaging, because fluorescence life-time is a much more robust read-out than fluorescence intensity [33]. Thus, FRET between the same type of fluorophore (homo-FRET) can be studied, which enables analysis of the oligomeric state of protein complexes *in vivo*. Protein interactions at the plasma membrane can be selectively monitored by total internal reflection fluorescence (TIRF) microscopy [41] (see below).

FRET-based techniques are limited by the relatively high background of cellular autofluorescence, as well as by the direct excitation of the fluorescence acceptor, which frequently biases the interaction experiments. These drawbacks are avoided by a technique called bioluminescence resonance energy transfer (BRET), which makes use of bioluminescence as the ‘energy donor’ [42,43]. Typically, Renilla luciferase is used as the BRET donor, whereas both GFP and YFP have been used as acceptors. This technique has been very successfully applied for demonstrating the oligomerization of G-protein-coupled receptors (GPCRs) [44,45] and for monitoring the activation state of receptor tyrosine kinases [46]. Detection of BRET is not possible by flow cytometry or microscopy, but requires very sensitive detectors. Its straightforward and rugged read-out (no excitation is required!), however, makes this technique better suited to high-throughput screening (HTS) than FRET.

It should be emphasized that the power of FRET and BRET techniques lies in the possibility of studying protein interactions in real time *in vivo*; however, these techniques are technically and experimentally rather demanding. For screening and identifying protein interactions, the previously mentioned two-hybrid reporter systems are more readily implemented and have a more robust read-out.

Identification of protein interactions *in vitro*

The identification of protein interactions *in vivo* requires validation *in vitro*, as a high number of false positive results are generated. Furthermore, interacting partners need to be characterized in more detail with respect to post-translational modification, cofactor or other proteins in the complex. For this task, *in vitro* methodologies are currently being established with high throughput and versatile read-out.

Tandem affinity purification

The classic biochemical techniques for detecting protein interactions *in vitro* are immunoprecipitation and pull-

down assays, both of which are based on affinity purification of a bait protein. These techniques were refined for proteomics applications by a technique called tandem affinity purification (TAP) [47,48]. TAP is based on an affinity tag that is used for two consecutive steps of affinity purification under very mild and selective elution conditions (Figure 2b). A bait protein tagged with the TAP tag is expressed in a target cell and subsequently purified by TAP. Thus, protein complexes involving the bait protein are purified, and are subsequently analyzed by SDS-PAGE and mass spectrometry. In a huge experimental effort, more than 200 distinct protein complexes in yeast have been identified, characterized and validated by TAP [49]. One key advantage of this technique is that only the bait is genetically modified with the affinity tag, while the whole proteome is 'fished' for prey. Furthermore, interactions between individual proteins and within entire protein complexes are being identified. However, transient interactions and complexes cannot be detected by TAP. TAP has been revealed to be particularly powerful when used in combination with Y2H [4[•],28].

Protein arrays

The concept of the protein array is the hallmark of functional proteomics and interactomics, as it is the obvious continuation of the DNA array approach. Different proteins are immobilized onto solid supports in a spatially resolved manner and the binding of interaction partners (or another protein function) is detected in parallel for each protein. Since the first attempts at highly multiplexed protein binding assays on surfaces [50,51], this field has witnessed tremendous development [52]. One of the most challenging aspects of this technology is the functional immobilization of a large number of different proteins with different physicochemical properties. Frequently, antibody arrays are used to capture proteins [53], but other techniques for direct protein immobilization, such as metal chelators [50] and streptavidin, have also been used. Typically, the read-out of protein arrays is indirect and uses fluorescence or chemoluminescent probes. However, direct detection by mass spectrometry [54] and label-free solid-phase detection (see below) are promising read-out strategies for protein arrays. Currently, protein arrays still require complex and expensive equipment for sample handling and detection. This technology, however, is developing rapidly, and has the potential to integrate the identification of interaction partners and detailed characterization of the interaction (see below).

Characterization of protein interactions *in vitro*

Numerous techniques for characterizing the interactions of purified proteins *in vitro* have evolved, and have been applied and further refined in recent years. These techniques are often specialized, and require rather large

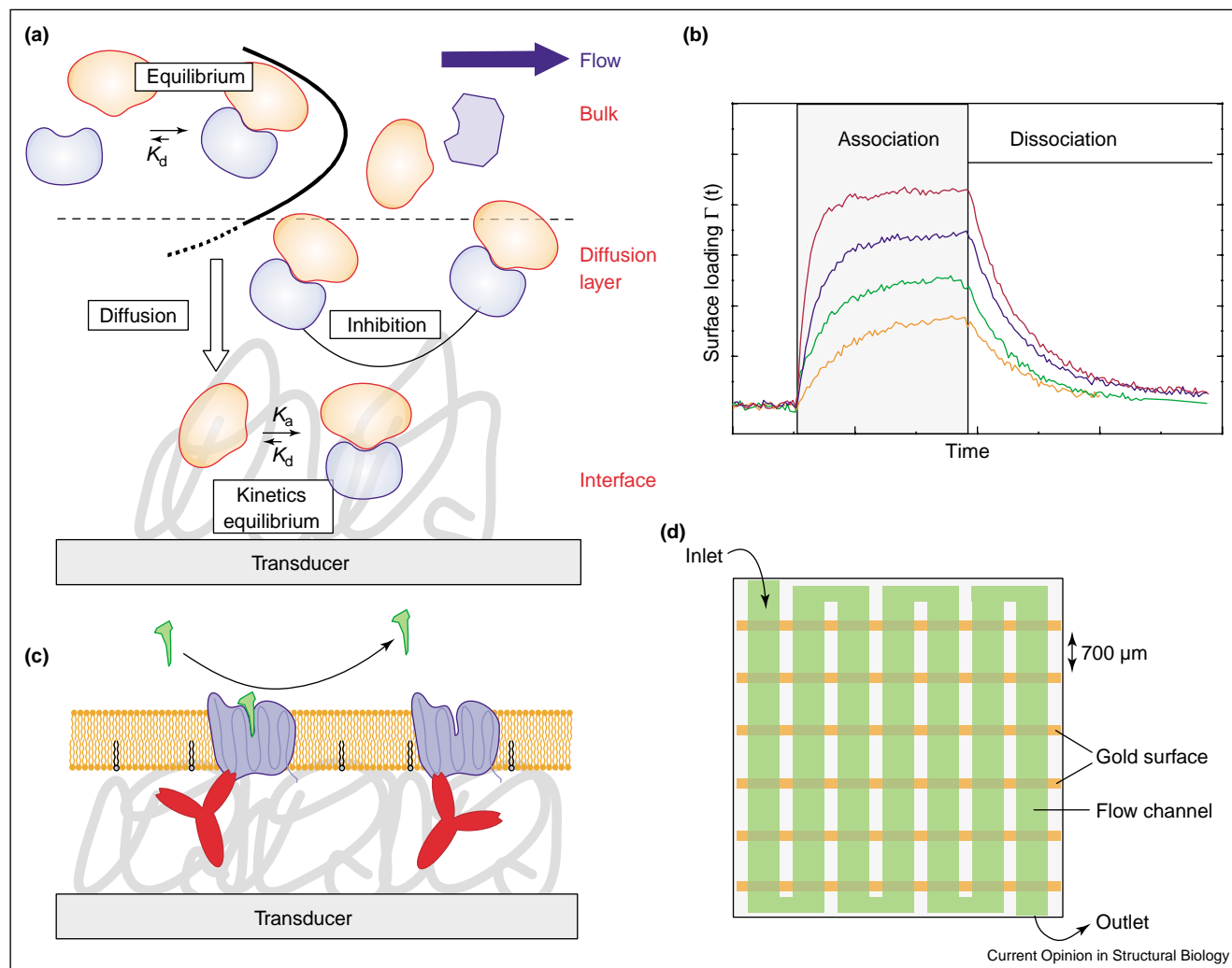
amounts of protein or elaborate labeling of the interacting partners with spectroscopic probes. In particular, fluorescent probes are frequently used, providing a versatile means for studying the kinetics, affinity and conformational changes of protein interactions [33]. Isothermal titration calorimetry is a powerful tool for the detailed thermodynamic analysis of protein interactions and has gained significant importance during the past few years [55]. Discussing all of these techniques is beyond the scope of this review. Here, I will focus on label-free solid-phase detection techniques, which can be applied in a very generic manner and are therefore particularly promising for genome-wide assays of protein interactions.

Solid-phase detection

Since the introduction of the BiacoreTM 'biosensor' system in the early 1990s, the label-free detection of protein-protein interaction at solid surfaces by surface plasmon resonance (SPR) and related techniques has gained tremendous popularity [56,57]. These techniques detect the interaction of a soluble ligand with a receptor immobilized on the surface of a physicochemical transducer (Figure 3a). One important advantage of these techniques is that no labeling of the interaction partners is required, but interactions can be characterized in great detail due to versatile assay formats. As interactions are detected in real time, both the equilibrium and the interaction kinetics can be analyzed, providing important and experimentally robust parameters for characterizing protein interactions (Figure 3b). Binding kinetics with rate constants ranging from 1 s^{-1} to $1 \cdot 10^{-4} \text{ s}^{-1}$ can be studied, and equilibrium dissociation constants between 100 pM and 100 μM can be quantified. As one of the interacting components needs to be immobilized, appropriate surface modification and tethering of proteins to these surfaces are central issues in the development of successful binding assays. In recent years, substantial progress has been made in this field, and numerous surface architectures and immobilization techniques are available [57]. Impressive progress was made in particular in the field of reconstituting membrane proteins in a functional manner on surfaces [58]. The capture and functional reconstitution of GPCRs onto such surfaces for studying ligand binding has been shown by SPR [59[•]] (Figure 3c).

A key advantage of solid-phase techniques lies in the heterogeneous assay format, which simplifies sample handling and minimizes sample consumption. Thus, solid-phase detection techniques potentially cope with today's need for multiplexing and automatizing binding assays for high-throughput analysis. Several optical detection techniques, such as SPR, reflectance interference (RIf) and ellipsometry, have been explored and optimized with respect to biomolecular interaction analysis over the past 15 years [60]. Although the application was, until recently, dominated by SPR [61], other signal transducers are gaining importance. In recent years,

Figure 3



Principles and applications of solid-phase detection. **(a)** Binding of a soluble ligand to an immobilized receptor is detected in real time either by label-free interrogation with an optical or acoustic transducer or by TIRF. Constant sample concentration is maintained by flow-through conditions. These techniques can be used to measure the kinetics and equilibrium of the interaction at the surface, as well as to probe equilibrium in solution by binding inhibition assays. **(b)** Typical binding curves obtained by solid-phase detection. The gray rectangle indicates the injection of the sample. Constant sample concentration is maintained by flow-through conditions **(c)** Reconstitution of integral membrane proteins, such as GPCRs (blue), in polymer-supported lipid bilayers for studying ligand binding (adapted from [59]). **(d)** Multiplexed solid-phase detection by SPR imaging of a peptide array attached to a gold surface using microfluidics (adapted from [66]).

label-free solid-phase detection has remarkably progressed towards highly parallel detection, which is particularly promising for protein array applications. Several approaches using SPR for imaging in an array format have been described [62–65] and several commercial systems are already on the market. Recently, protein interaction with a peptide array monitored by imaging SPR with impressive time resolution and signal-to-noise ratios has been reported [66*] (Figure 3d). Other optical techniques have been used for parallel label-free detection of protein interactions. RIF was successfully applied to parallel ligand binding assays [67] as well as epitope mapping [68]. Interaction screening was recently reported using a

novel system based on integrated optics (BIND) [69]. However, whereas detectors easily cope with massive parallelization, multiplexed protein immobilization and sample handling are currently limiting the throughput of these systems. For their successful implementation, generic and robust approaches for the site-specific immobilization of proteins, as recently reported [70], will be important.

The versatility of label-free detection techniques has been further enhanced by combination with other techniques. Mass spectrometry has been frequently used in combination with SPR detection for the identification of

ligand specifically bound to immobilized proteins [71–76], either after elution and recovery of the specifically bound ligand, or directly from the surface using MALDI-MS (mass spectrometry). Using these methods, ligands have been identified from cell extracts [77] and tissue extracts [78]. Thus, the single-step identification and characterization of protein interaction can be envisaged. Furthermore, label-free detection was combined with surface-sensitive fluorescence detection. Generic and quantitative mass-sensitive detection, which is inherently prone to high background and noise, is efficiently complemented by highly selective and sensitive fluorescence detection, with its wide-ranging methodological repertoire [33]. SPR has been combined with TIRF spectroscopy, making use of evanescent field enhancement by the surface plasmon [79]. Recently, RIf combined with TIRF spectroscopy was applied to dissecting the mechanism of ligand-induced receptor assembling on supported lipid bilayers [80•].

Emerging techniques: single-molecule detection

The past decade has witnessed a stunning development in methodologies for studying biomolecular interactions on the single-molecule level. Apart from the pure fascination of watching individual molecules in action, there are some distinct advantages of these techniques over conventional ensemble techniques: interaction assays can be carried out with very low quantities and concentrations, which often correspond to the natural cellular level; functional inhomogeneity is not averaged and therefore can be identified; and the dynamics of interactions at equilibrium can be studied. Single-molecule detection is often carried out on highly defined samples *in vitro*; however, these techniques are particularly promising for visualizing protein interactions *in vivo* and exciting studies have already been published (see below). Currently, two main approaches for studying protein interactions on the single-molecule level have been followed: atomic force microscopy (AFM) and fluorescence techniques.

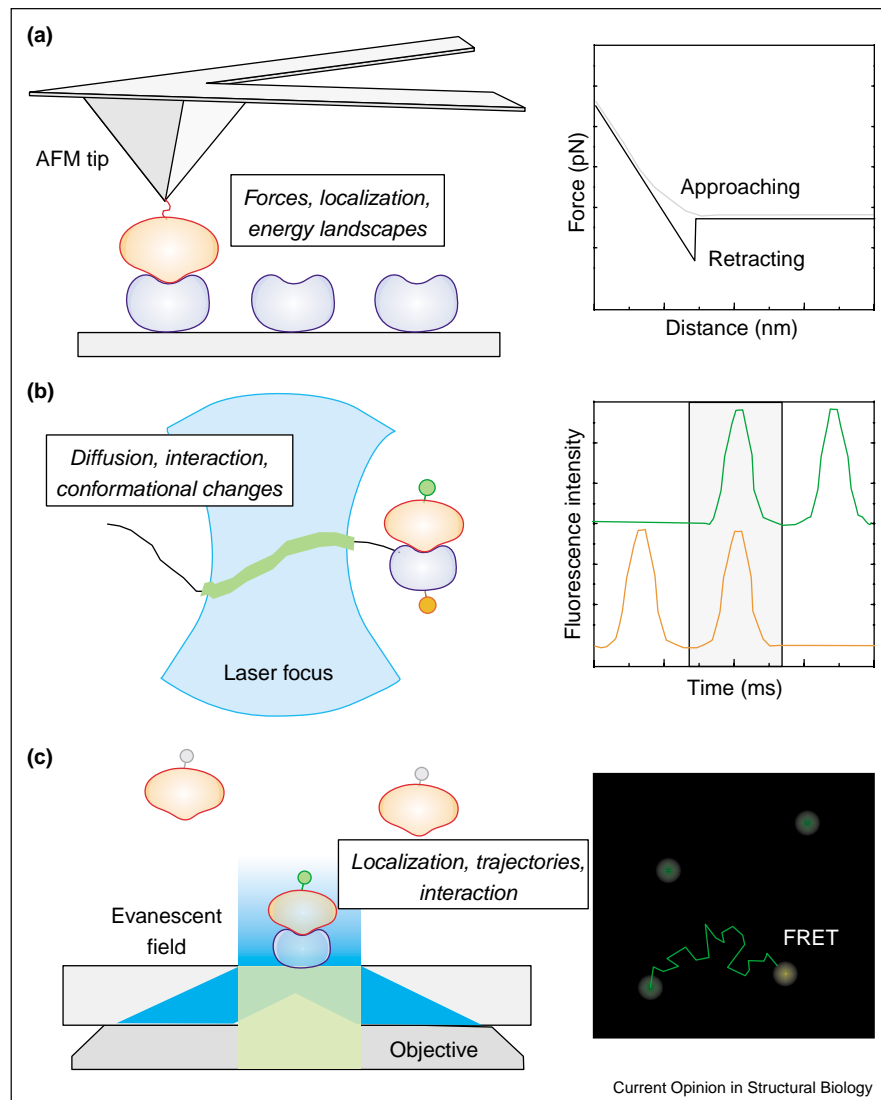
AFM has opened a new window towards characterizing the forces involved in protein interaction using a technique called force spectroscopy [81]. One of the interacting partners is tethered to the tip of the AFM, while the other is immobilized on a surface (Figure 4a). Upon interaction, the forces required to retract the tip from the surface increase, which can be measured very accurately. Although interaction forces are intuitively less meaningful than interaction energies, force/distance relationships can provide valuable information on the reaction coordinate of interactions [82••].

Several fluorescence detection approaches have been successfully applied to probing protein interactions on the single-molecule level *in vitro* and in living cells

(recently reviewed in [83]). Fluorescence correlation spectroscopy is a powerful method for studying the interaction dynamics of protein complexes in solution. Fluorescence fluctuations in a very small confocal volume are detected, from which the diffusion characteristics of individual molecules can be analyzed (Figure 4b). Interactions can be detected as changes in diffusion properties. Fluorescence correlation spectroscopy is currently most suitable for dissecting the dynamics of proteins and protein interactions *in vitro*. Its application *in vivo* is limited because the concentrations and diffusion coefficients of the analyzed species have to be tightly controlled. A more robust approach for studying protein interactions was realized by analyzing fluorescence cross-correlation of proteins labeled with different fluorophores. Upon interaction of these species, the probability of simultaneous diffusion through the confocal volume is highly increased, which is detected by cross-correlation of the emission signal of the two fluorophores [84]. This technique is particularly promising for dissecting complex interactions (e.g. interaction with multiple partners). The application of fluorescence cross-correlation spectroscopy to monitoring interactions in living cells has been demonstrated [85], even for interactions between autofluorescent proteins [86]. Confocal fluorescence detection can also be used for measuring protein interactions on the single-molecule level by FRET. Using confocal single-molecule fluorescence spectroscopy, conformational fluctuations in proteins and protein complexes could be monitored [87,88].

The imaging of interactions at interfaces on the single-molecule level was achieved by TIRF microscopy (c.f. Figure 4c) [89]. This technique was very successfully used to study motor proteins *in vitro* [90]. During recent years, it was increasingly applied to visualizing signal transduction on the molecular level in living cells [91]. Ligand binding to its receptor [92] and effector recruitment at the plasma membrane [93] have been studied. One particular advantage of single-molecule imaging is the possibility of lateral localization with an accuracy way beyond the refraction limit [94], which can be used for high-resolution single-particle tracking. Simultaneous tracking and interaction analysis experiments are particularly useful for studying the early steps of signal activation at the plasma membrane [93,95••]. However, single-molecule imaging *in vivo* is also feasible by epifluorescence detection. Recently, the binding of a model substrate to the nuclear pore complex and its translocation into the nucleus were monitored in permeabilized cells with millisecond time resolution [96]. A serious limitation of fluorescence-based single-molecule imaging, however, is the relatively fast photobleaching of organic fluorophores, which typically limits the observation time for an individual fluorophore to a few seconds. Therefore, these techniques are currently most suitable for studying processes on this timescale. Protein–protein

Figure 4



Single-molecule detection techniques. **(a)** Force spectroscopy with a ligand attached to an AFM tip. During the approach of the tip, the repulsion force increases with decreasing distance; this is inverted during retraction. An additional force is required to fully retract the tip because of the interaction with the immobilized receptor. Upon rupture of the interaction, the force jumps to the baseline. **(b)** Several non-imaging fluorescence techniques are based on confocal detection in a very small volume. From the bursts in fluorescence intensity during passage through the confocal volume, the diffusion and dynamics of proteins and protein complexes can be studied. Simultaneous two-color detection allows the analysis of protein complexes by cross-correlating the fluorescence bursts (gray area). **(c)** Single-molecule imaging by TIRF microscopy visualizes individual molecules, which can be localized way below the refraction limit by analyzing the lateral intensity distribution (point spread function). Thus, high-resolution trajectories can be determined, combined with interaction analysis by FRET.

interactions often have much longer life-times and therefore cannot be analyzed. Recent developments in fluorescent nanocrystal technology (quantum dots) may prove important for extending the applications of single-molecule fluorescence imaging [93].

Concluding remarks

Tremendous developments in the field of protein interaction analysis have been fueled by current efforts in the life sciences to dissect cellular protein function. I have

attempted to cover the spectrum of techniques for identifying and characterizing protein interactions, ranging from biochemical methods in the cellular context and technological protein array approaches to advanced biophysical techniques for studying isolated individual protein molecules under well-defined conditions (Table 1). Fully understanding the function of proteins and protein networks in the cell requires approaching protein interaction from these many different perspectives. Protein scientists have to broaden their view and face a 'fusion of

Table 1

Features and applications of different techniques for protein interaction analysis.

Technique	Application	Identification	Localization	Affinity	Kinetics	Throughput	Membrane proteins	K_d range
Yeast two-hybrid	<i>In vivo</i>	++	–	–	–	++	–	<50 μ M
Protein fragment complementation	<i>In vivo</i>	+	–	+/-	+/-	+	+	<10 μ M
Tandem affinity chromatography	<i>In vitro</i>	++	+/-	–	–	++	+/-	<50 nM
Protein arrays	<i>In vitro</i>	+	+/-	+	+/-	++	–	<1 μ M
FRET/BRET	General	+/-	++	+	+/-	+/-	+/-	<10 μ M
Solid-phase detection	<i>In vitro</i>	+/-	–	++	++	+	+	pM– μ M
Single-molecule detection	General	–	++	++	++	+/-	+	nM– μ M

disciplines' [97] on these levels; many experiments can be done by many specialists, but in the end someone has to pull together all these strings to create a model of how proteins work in the cell.

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