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Deciphering complex protein interaction kinetics using Interaction Map

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ABSTRACT

Cellular receptor systems are expected to present complex ligand interaction patterns that cannot be evaluated assuming a simple one ligand:one receptor interaction model. We have previously evaluated heterogeneous interactions using an alternative method to regression analysis, called Interaction Map (IM). IM decomposes a time-resolved binding curve into its separate components. By replacing the reductionistic, scalar kinetic association rate constant k_a and dissociation rate constant k_d with a two-dimensional distribution of k_a and k_d, it is possible to display heterogeneous data as a map where each peak corresponds to one of the components that contribute to the cumulative binding curve. Here we challenge the Interaction Map approach by artificially generating heterogeneous data from two known interactions, on either LigandTracer or Surface Plasmon Resonance devices. We prove the ability of IM to accurately decompose these man-made heterogeneous binding curves composed of two different interactions. We conclude that the Interaction Map approach is well suited for the analysis of complex binding data and forecast that it has a potential to resolve previously uninterpretable data, in particular those generated in cell-based assays.

1. Introduction

The biophysical characterization of protein–protein interactions by use of time resolved analyses is a wide field of studies where interactions of isolated molecular components are commonly characterized using Surface Plasmon Resonance (SPR) or Quartz Crystal Microbalance (QCM) devices. Recently, novel tools for time-resolved analysis of the interaction of molecules with cells are emerging, of which LigandTracer is one. For isolated components the common basic assumption in data evaluation is that the binding curves conform to a one:one interaction. Experimental curves from cell-based assays, and also from some SPR/QCM assays, contradict the one:one binding assumption.

The fundamental problem of protein–protein interaction analysis on cells or tissue lies in the complexity of cell surfaces. A protein is likely to interact not with one form of the intended receptor at the cell-surface, but with variants of it, such as post-translationally modified receptors, activated/inactivated forms or other conformers. For example the receptor may dimerize with itself or with homologues. Interactions with completely different cell-surface-associated proteins are possible as well. One example is the EGF-EGFR interaction, which has been thoroughly analyzed and discussed during the last three decades [1–3] and which commonly results in curvilinear Scatchard plots [4]. EGFR is known to form both homodimers and heterodimers with the other members of the EGFR family (i.e. ErbB2, ErbB3, and ErbB4), and ligand-induced dimerization is necessary for activation of EGFR [5].

Each binding event may have its own set of interaction parameters. Hence it is difficult or even wrong to approximate a protein-cell interaction with reductionistic interaction models. Complex situations may also occur in the analysis of isolated components due to e.g. repeated subunits within a protein or conformational heterogeneity due to purification or surface immobilization. Current methods that are applied for the evaluation of binding curves are insufficient because they are based on regression analysis using models with limited complexity. The development of novel analysis tools, able to detect and unravel interaction complexities, is obviously required.

A method for analyzing the complexity resulting from parallel interactions in isolated molecular systems was originally developed by Svitel and co-workers [6–8]. It has been applied to probe the degree of homogeneity of the receptor population in cell-free

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protein–protein interaction analysis [6,8] to determine affinity and dissociation rates [9], and to elucidate binding mechanisms [10,11]. We have previously extended the approach to the much more complex situation of a ligand binding to living cells [3,12,13] as well as to biophysical interaction data [13].

The method of Svitel et al. [7] is well adapted for quantification of binding properties for one relatively pure component (e.g. the protein) binding to one heterogeneous component (e.g. the receptor population on the cell). The key element in this analysis lies in the use of a two dimensional distribution of interaction parameters, wherein it is assumed that each interaction of a protein in solution with a protein on a cell is monovalent. In our implementation, the distribution of interaction parameters is made discrete by creating a grid of k_a and k_d values. Each $(k_a, k_d)_{ij}$ node has a corresponding primitive interaction curve and a magnitude B_{ij}, and a heterogeneous measurement is approximated with a sum of all primitive curves scaled with the accompanying B_{ij}. Given one or more measured curves, the magnitudes of all B_{ij} can be fitted and then plotted in a surface plot as a function of k_a and k_d. We denote this plot an Interaction Map (IM) for display of interaction characteristics and heterogeneity.

While these approaches have been applied to identify binding complexity and resolve associated parameters, they have never been validated with known heterogeneous interactions systems, to the best of our knowledge. In this paper, we challenge the *Interaction Map* method by evaluating man-made heterogeneous kinetic curves generated in two ways: by injecting a protein on two co-immobilized binding partners in a Biacore Surface Plasmon Resonance (SPR) device, and by mixing two independent protein–protein interactions in a LigandTracer device.

2. Materials and methods

2.1. SPR-based protein interaction experiments

Data for the interaction of Fab57P [14] with peptides derived from the sequence of toabacco mosaic virus protein, were collected at 25 °C on a Biacore 2000® instrument (GE-Healthcare Biacore, Uppsala, Sweden) as previously described [15], except that the running buffer contained 250 mM instead of 150 mM NaCl (Hepes250:10 mM HEPES, 250 mM NaCl, 3.4 mM EDTA, 0.005% Tween 20, pH 7.4). The peptides correspond to residues 138–149 (P138–149) and 140–151 (P140–151) of the protein, with a N-terminal cysteine for surface immobilization (sequences CSYNRSSFESSSG and CNRSSFESSSGLV, respectively). Peptides were immobilized one per flow cell or two per flow cell (in 25%/75% ratios).

2.2. LigandTracer-based protein interaction experiments

105 μg trastuzumab (purified from Herceptin, Roche AB, Stockholm, Sweden) and 40 μg human serum albumin (HSA, Sigma Aldrich, St. Louis, MO) was labeled with 10 MBq ^{125}I (Perkin-Elmer, Wellesley, MA, USA) using the established Chloramine-T protocol [16]. Prior to each LigandTracer measurement, 2 μl Protein A coated magnetic beads (1.3 g/ml) (Dynabeads, Dynal A.S, Oslo, Norway), was incubated with 6 μg of the HSA binding monoclonal antibody 18080 (Abcam, Cambridge, MA, USA) in 100 μl PBS for 60 min on rotation. The magnets were washed three times with 200 μl PBS to remove excess antibody.

The interaction of ^{125}I-HSA with 18080 and/or ^{125}I-trastuzumab with Protein A was monitored in BSA pre-coated polystyrene dishes (Cat. No. 254925, Nunc, Roskilde, Denmark) using LigandTracer Grey (Ridgeview Instruments AB, Uppsala, Sweden), essentially as described previously [17]. The binding of a stepwise increase of concentration (7.8 and 24.3 nM in PBS + 1% BSA) was monitored for ^{125}I-HSA, ^{125}I-trastuzumab and a combination of ^{125}I-HSA and ^{125}I-trastuzumab (7.8 and 24.3 nM of each) during 4 h for each concentration, followed by a dissociation measurement over-night. The contribution of ^{125}I-trastuzumab and ^{125}I-HSA to the measured binding curve were altered by using ^{125}I-trastuzumab with different specific activities (numbers of radioactive nuclides per protein molecule).

2.3. Simulations

Binding curves matching the concentrations and times used for both SPR and LigandTracer experiments were simulated using the one:one interaction model for a range of different affinities and kinetics using TraceDrawer (Ridgeview Instruments AB, Uppsala, Sweden) and MATLAB 6.5 (The Mathworks Inc, MA, US).

2.4. Interaction Map generation

Under the assumption that each interaction contributing to the complex interaction can be described as a one:one interaction, the measured binding curve will be a sum of all individual one:one interactions. The measured curve can be approximated with a sum of a range of primitive binding curves, each representing a one:one interaction [3] with a unique combination of association rate k_a and dissociation rate k_d (and consequently an equilibrium dissociation constant $K_D = k_d/k_a$).

$$EstimatedCrv = \sum_{i=1}^{n}\sum_{j=1}^{m}\left[B_{ij} \times \frac{conc}{conc + k_d^i/k_a^i} \times PrimitiveCrv(conc, k_a^i, k_d^i)\right] \quad (1)$$

The constants B_{ij} represent the contribution of each primitive binding curve *PrimitiveCrv* to the estimated curve *EstimatedCrv*, and reflects the surface concentration of the corresponding receptor. There are $n \times m$ *PrimitiveCrv* with kinetic constants (k_d^i, k_a^j). Given one or more measured curves, the magnitudes of all B_{ij} were estimated by use of a non-linear fitting algorithm and were then plotted in a surface plot as a function of k_a and k_d. This plot is denoted an *Interaction Map* (IM). The surface plot may be presented in grey-scale (black = large B_{ij}, white = small B_{ij}). There are several computational issues with such an approach. Firstly, it is essential that a sufficient number of k_a and k_d values (and accompanying primitive curves) are used for representing the two-dimensional distribution of k_a and k_d, otherwise this method will have poor resolution when decomposing a measured binding curve. Secondly, it is essential to use regularization algorithms to suppress non-physical solutions to the fitting problem. Thirdly, the choice of non-linear fitting algorithm will be crucial to obtain accurate fits in reasonable time. The currently used Interaction Map method uses 24 $(k_a) \times 30$ (k_d) different nodes (with accompanying primitive curves) with kinetic parameter values evenly distributed in log-space. For interaction measurements longer than 1 h the kinetic parameter values were ($\log10(k_a) = \{2.00, 2.25, \ldots, 7.50, 7.75\}$, $\log10(k_d) = \{-6.60, -6.40, \ldots, -1.00, -0.80\}$). For interaction measurements shorter than 1 h the kinetic parameter values were ($\log10(k_a) = \{2.00, 2.25, \ldots 7.50, 7.75\}$, $\log10(k_d) = \{-5.60, -5.40, \ldots, 0.00, 0.20\}$). A Tichonov-type regularization algorithm was used, which in practice adds penalty to the sum-of-square residuals if there are many peaks in the Interaction Map. The non-linear fitting algorithm as currently implemented in Visual Basic/Visual Studio 2005 (Microsoft Inc, Mountain View, CA, USA) is stable but slow, resulting in 3–30 h computation time per map on a regular PC.

3. Results

In a first experiment, the IM method was applied to experimental SPR data. Heterogeneous data were created by injecting Fab57P