Transmembrane Signalling of the Insulin Receptor: 3D Reconstruction from STEM Imaging, Crystallography and NMR Spectroscopy.

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One of the major goals in molecular biology is the understanding of the relationship between the 3D structure of macromolecules, alone or in complex assemblies, and the biological function or mechanism of action that ensues as the result of that structure. To achieve that goal, the need for structural detail at atomic resolution has been a tacit assumption. Almost by definition such a requirement eliminates the use of electron microscopy (EM) of biological specimens, with a concentration instead on x-ray crystallography and, more recently, nuclear magnetic resonance (NMR)spectroscopy.

Nevertheless, EM and electron diffraction of thin monomolecular crystals, although not yet providing atomic resolution directly, have resulted in 3D structures with about 3.5 Å detail [1,2]. However, the *forte* of EM is its capability to image individual macromolecules and complexes which have been refractory to crystallization and which are too large to be solved by NMR spectroscopy. The spatial resolution for 3D reconstructions of large macromolecules or complexes from such single particle imaging is generally between 10 and 20 Å. However, when subdomains of such macromolecules have been solved by crystallography or NMR, such domains can be docked or fitted into the EM reconstruction to much greater accuracy using the constraints of the centre of mass of the subdomain and its EM counterpart, the envelope of that part of the EM reconstruction constraints, and the limitations to possible rotation due to the asymmetry of the structure.

This combination of techniques has been used by us to result in the virtually complete atomic structure of the 480 kDa insulin receptor in the presence of insulin [3,4], resulting in an understanding of the binding of insulin ligand at the level of amino acid side chain interactions. Moreover, the connectivity of the structural domains and their three-dimensional spatial juxtaposition led to the prediction of the mechanics of transmembrane signalling and activation of this tyrosine kinase membrane receptor. This prediction has been substantiated by our current reconstruction of the insulin-free receptor (Fig. 1).

Technically, most single particle EM approaches use bright field phase contrast imaging. In contrast, we have developed and exploited low-dose, low-temperature dark field techniques using scanning transmission electron microscopy, to take advantage of the high contrast of that procedure which permits easy visualization of freeze-dried macromolecules as small as a few tens of kilodaltens. Imaging doses as low as 2 e/Å^2 were made possible via the simultaneous signal acquisition of elastic and inelastic electron scatter signals from the specimen at each picture point delineated by the 3 Å beam. Three-dimension reconstruction was carried out using by filtered back-projection after deriving the relative 3D orientations of individual molecular images by reference-free iterative quaternion-assisted angle determination [5,6].

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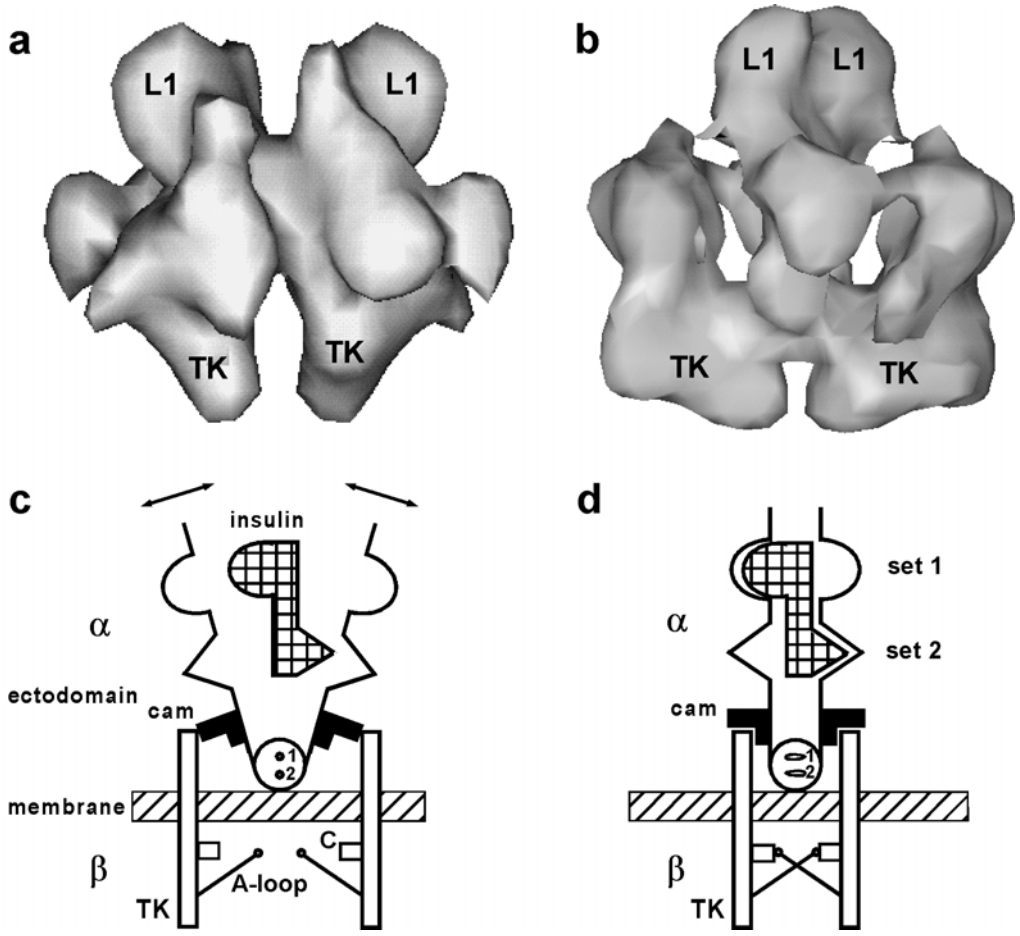

Fig. 1 Structure and Mechanism of Transmembrane Signalling and Activation of the Intrinsically Dimeric Insulin Receptor
(a) 3D reconstruction of the insulin-free receptor. (b) 3D reconstruction of the insulin receptor with insulin bound. (c) Schematic of the structure of the insulin-free receptor showing the structural relationship between the extracellular α-subunits and the transmembrane β-subunits of the receptor. (d) Schematic of the structure of the insulin-bound receptor. L1 = large domain 1; TK = tyrosine kinase domain; C = catalytic site; A-loop = activation loop; 1,2 = covalent cys-cys bonds between receptor monomers; cam = specific structural feature; set 1,2 = specific sets of binding side chains on α-subunits.

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