Larger and longer: atomistic and coarse-grained simulations of the Epidermal Growth Factor Receptor

In humans, the epidermal growth factor receptor (EGFR) is one of a family of four cell receptors governing cell proliferation, differentiation, and death. The associated signalling network is implicated in the development of most human cancers and is one of the main targets of anti-cancer therapeutic agents.

Structurally, the EGFR protein consists of: (i) an extra-cellular domain (ectodomain), composed of four distinct sub-domains; (ii) a single trans-membrane alpha-helical region; and (iii) a cytoplasmic catalytic region, comprising the tyrosine kinase domain, flanked by the juxtamembrane and C-terminal regulatory regions. Crystallographic data are now available for several topologies of the ectodomain, and for the tyrosine kinase domain.

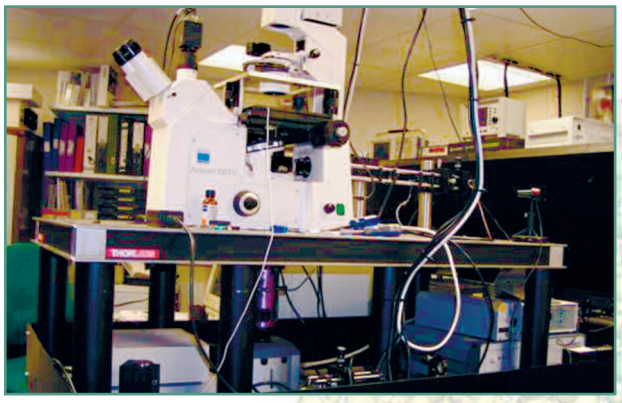

Figure 1: The Advanced Single Molecule Imaging and Dynamics (ASMID) facility.

The ectodomain is able to bind a range of growth factor ligands and is thought to undergo a conformational change on binding from a "tethered" auto-inhibited configuration to an extended configuration that favours receptor "back-to-back" dimerisation. Ectodomain dimerisation leads to juxtapositioning of the associated intracellular kinase domains, their allosteric activation and phosphorylation at tyrosine residues in the juxtamembrane and kinase domains as well as in the C-terminal tail. These events trigger a string of responses leading to modified cellular behaviour through a series of secondary events that are typically initiated through the direct docking of effectors and scaffolds at appropriate phosphotyrosine residues.

Despite this simple model of EGFR activation, the reality appears to be more complex. Recent fluorescence experiments on the STFC ASMID facility (Figure 1) have revealed a separation between bound ligands smaller than can be achieved by the back-to-back dimer model [1].

These experiments also reveal that the ectodomains do not always stand proud of the membrane, as is usually assumed. Experiments by several groups also suggest that EGFR can form higher order oligomers. Finally, these properties of the ectodomain differ markedly between the high-affinity and low-affinity forms of EGFR.

The region of the ectodomain closest to the membrane is only resolved by crystallography in the tethered conformation, and is presumably highly flexible in the untethered monomer and in the receptor dimer. This flexibility may facilitate novel receptor configurations, and receptor-receptor interactions. Molecular dynamics is ideally suited to reveal how the tertiary and quaternary structure of the receptor behaves in a variety of situations, relevant to the biological function. We have therefore performed a series of simulations on the EGFR system, with the aim of understanding the structure and dynamics of the receptor and how it relates to the fluorescence measurements.

Although we are currently focussing attention on the ectodomain region of the receptor only, even this, when taken with the associated lipid membrane and surrounding water, constitutes a large simulation. We have simulated systems with several hundred thousand atoms, with the largest system including 840,000 atoms. Atomistic systems have generally been simulated using NAMD 2.6, with some control simulations using Amber version 9. The former used CHARmm all-atom force fields, while the latter used the AMBER/ff03 force field. Simulations were carried out on the IBM Blue Gene/L and Blue Gene/P systems at STFC Daresbury Laboratory, and on the HECToR national service. Figure 2 shows the scaling achieved by NAMD on HECToR for the largest system attempted.

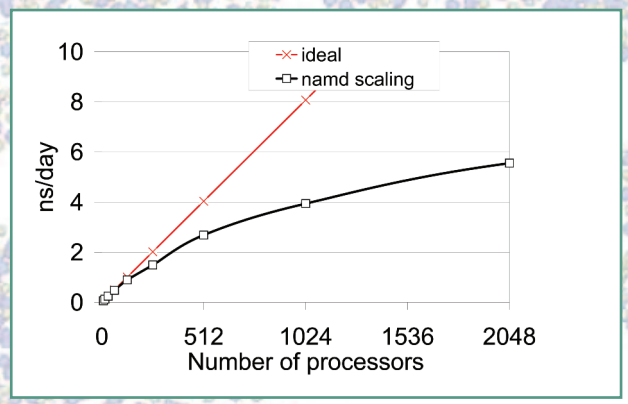

Figure 2: Scaling of NAMD on HECToR for the atomistic simulation of the EGFR tetramer on the membrane (840,000 atoms). The number of nanoseconds simulated per day is shown.

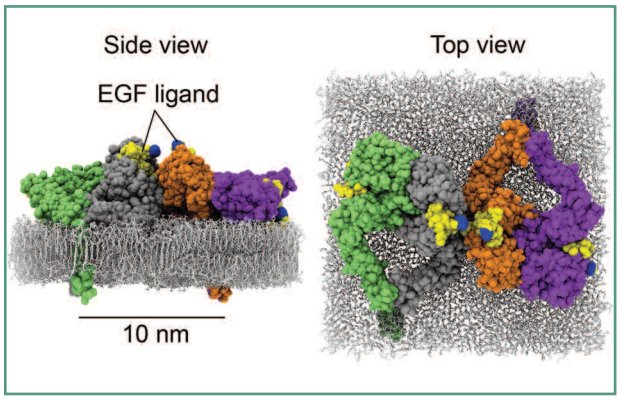

Figure 3: EGFR spans the plasma membrane of human cells, transducing the signal of growth factors. Atomistic simulations show a possible tetrameric arrangement of receptors on the cell surface.

Experiments have shown that EGFR ectodomains lie down such that the bound ligand is brought close to the membrane. From a structural point of view, however, the ectodomain is bulky and it is not clear to what extent this is possible. Atomistic simulations of the dimer placed lying down on the membrane revealed a domain rearrangement which facilitates a close approach, while preserving many of the features of the back-to-back dimerisation.

To explain the small separation between pairs of bound ligands seen in fluorescence experiments, we had previously identified a "head-to-head" interaction between receptors, seen as a crystal contact in one structure [1]. It was unclear whether this interface would be stable outside the crystal environment, i.e. whether it would be biologically relevant. Atomistic simulations showed a relaxation of the interface with respect to the crystal structure which creates a larger interface, with a number of stabilising interactions.

Experiments have also suggested the possibility of receptor tetramers, with one candidate being two back-to-back dimers joined by a head-to-head interface. Simulations showed that such tetramers are indeed geometrically feasible in the membrane environment, and stable at least over the timescale of the simulation (30 ns), see Figure 3.

Large biological systems can also be addressed by simplifying the representation of the macromolecules, and coarse-grained (CG) models are gaining popularity. Marrink's group in Groningen have developed the MARTINI force field [2], in which coarse-grain particles represent on average 4 heavy atoms. We have used the MARTINI force field to develop a model for EGFR ectodomains in a lipid membrane environment.

Using the CG model, we have run simulations of ectodomain monomers, dimers and tetramers, together with the connected trans-membrane helices and the lipid

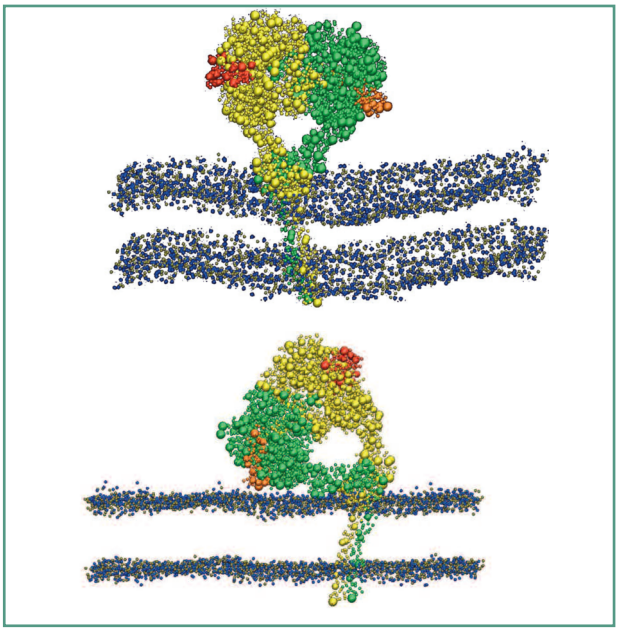

Figure 4: Coarse-grained simulations of EGFR on the cell membrane, showing a partial lying down of a receptor dimer. Simulations of a monomer have captured a complete collapse onto the membrane surface (not shown).

bilayer. The ectodomains show large flexibility, especially in the sub-domain closest to the membrane. In particular, the ectodomains can approach the membrane rather easily, supporting the ideas behind the atomistic simulations (Figure 4). The timescale for this diffusive motion is of the order of 500 ns, i.e. outside the range of present-day atomistic simulations.

In conclusion, with available HPC resources we can simulate large membrane receptor systems, allowing us to rationalise and guide experiments. Use of CG parameterisations allows us to push the length and timescales significantly further. Switching between the two types of simulation will allow us to combine the ability of CG to explore slow conformational changes and protein motions with the detail achievable with atomistic simulations.

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References:

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3. http://www.cse.scitech.ac.uk/ccp4/compu tational_biology/