Final report for IEF fellowship " EphR signaling"

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Proteins in cell-to-cell communication: the Eph receptors and their ephrin ligands

Erythropoetin-producing hepatoma (Eph) receptors are cell surface protein tyrosine kinases mediating cell-cell communication. They direct key processes during development and repair of the nervous system, blood vessel formation, insulin secretion, immune system function, intestinal homeostasis and bone tissue integrity. Upon binding to their ligand, activated Eph receptors form signalling clusters. We set out to study the architecture of such clusters and solved crystal structures of the full ectodomain of human EphA2 (eEphA2), alone and in complex with the receptor-binding domain of the ligand ephrinA5 (ephrinA5^{RBD}). Unliganded eEphA2 forms linear arrays of staggered parallel receptors involving two patches of residues conserved across A-class Ephs. eEphA2-ephrinA5^{RBD} forms a more elaborate assembly, whose interfaces include the same conserved regions on eEphA2, but re-arranged to accommodate ephrinA5^{RBD}. Cell surface expression of mutant EphA2s demonstrated that these interfaces are critical for localization at cell-cell contacts and activation-dependent degradation. Our results suggest a 'nucleation' mechanism whereby a limited number of ligand-receptor interactions seed an arrangement of receptors which can propagate into extended signalling arrays.

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Our subsequent work on has extended these studies to additional ephrin-Eph complexes and is currently at the stage of functional analysis to validate proposed molecular mechanisms in the cellular context.

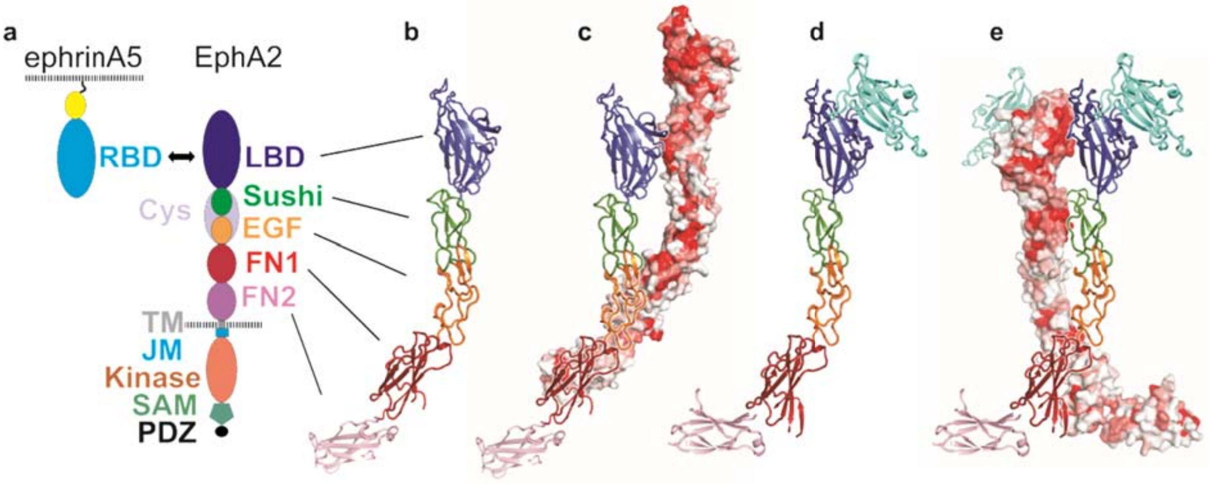

Figure 1| Crystal structures of the complete EphA2 extracellular region (eEphA2) alone and in complex with ephrinA5^{RBD}. a, Domain composition of ephrinAs and Eph receptors. **b,** Crystal structure of eEphA2 comprising EphA2 LBD, Cys, FN1 and FN2 domains. Colours are as in a. The previously uncharacterized Cys domain is composed of a sushi domain (CCP) and an EGF domain. **c,** Arrangement of two eEphA2 molecules within the crystal. Their contacts bury 2661 Å^2 of molecular surface. The front molecule is displayed and oriented as in a. The back molecule is shown in surface view and coloured according to human Eph class A receptor sequence conservation (red = strongest conservation, white = weakest conservation). **d,** eEphA2 (colours as in a) in complex with ephrinA5^{RBD} (cyan). The orientation of the sushi domain is the same as in b and c. **e,** Arrangement of neighbouring complexes within the crystal. The eEphA2 molecule on the right is oriented and coloured as in d. Its neighbour on the left is in surface view and coloured according to EphA2 sequence conservation. Their contacts bury 1240 Å^2.

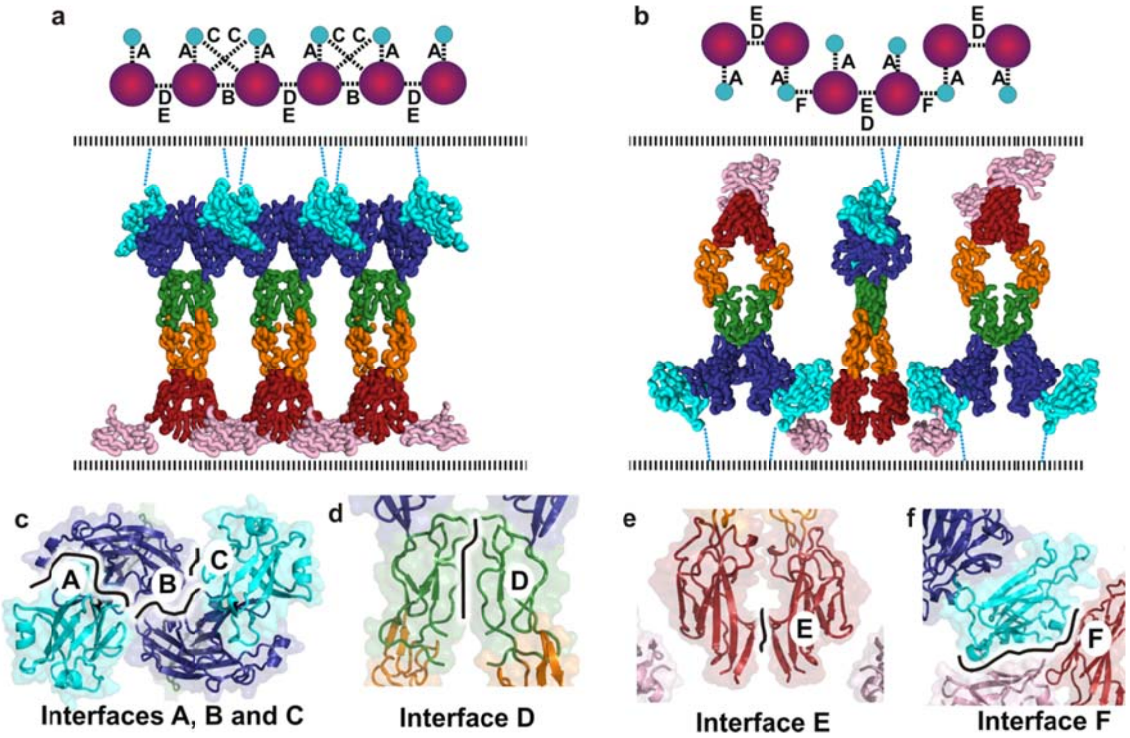

Figure 2 | The eEphA2-ephrinA5^{RBD} complex: structural changes within eEphA2 and formation of array-like clusters. a, b, Crystal packing of eEphA2-ephrinA5^{RBD}. The ribbon diagrams are coloured as in Fig. 1. The cartoon above each panel illustrates the crystal contacts designated interfaces A-F (dotted lines) for eEphA2 (purple) and ephrinA5^{RBD} (cyan). Panel a shows six symmetry-related eEphA2-ephrinA5^{RBD} complexes forming a parallel array; view is approximately along unit cell axis c. The protein arrangement reflects EphA2 and ephrinA5 interaction *in trans*, i.e. where the proteins are located on opposing cells. Panel b shows six symmetry-related eEphA2-ephrinA5^{RBD} complexes forming an anti-parallel array, view as in f, but tilted by ~45° around unit cell axis a. The arrangement shows EphA2 and ephrinA5 interacting both *in trans* and *in cis*, consistent with both proteins presented on both cells. **c-f,** Interfaces A-F are shown as black lines. Colours are as in a. Interfaces A-E form the crystal contact interactions shown in a, interface F is a major interface but contributes only to the contacts shown in b.

Figure 3 | EphA2 localization at cell-cell contacts depends on ectodomain clustering. Representative results are presented of functional studies using HEK293T cells. A full set of results is provided in Supplementary Fig. 6. Filled white arrows indicate cell-cell contacts between transfected cells. Open arrows point to cell-cell contacts between a transfected cell and a non-transfected cell. **a-c,** mVenus-tagged EphA2 accumulates at cell-cell contacts of transfected cells. **d-e,** Mutants in interfaces A, B, D and, to some extent, interface F do not cluster at cell-cell contacts of transfected cells but are distributed evenly on the cell surface (the interface B mutant G131Y is shown as an example). **a, d** Fluorescence images are shown. **b, e,** Phase contrast images of the corresponding cells. **c, f,** Overlay images of a, b, d, e.