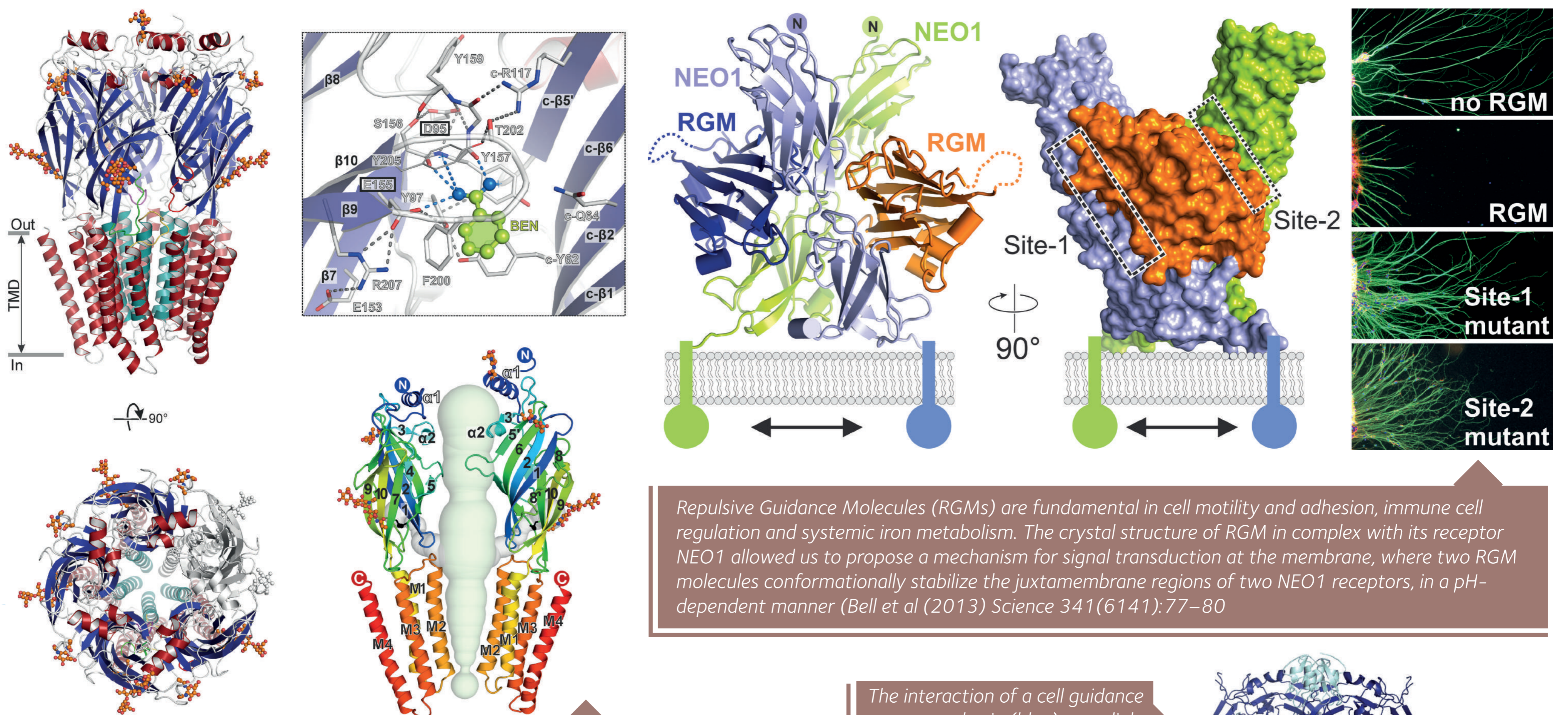


The Wellcome Trust Centre for Human Genetics

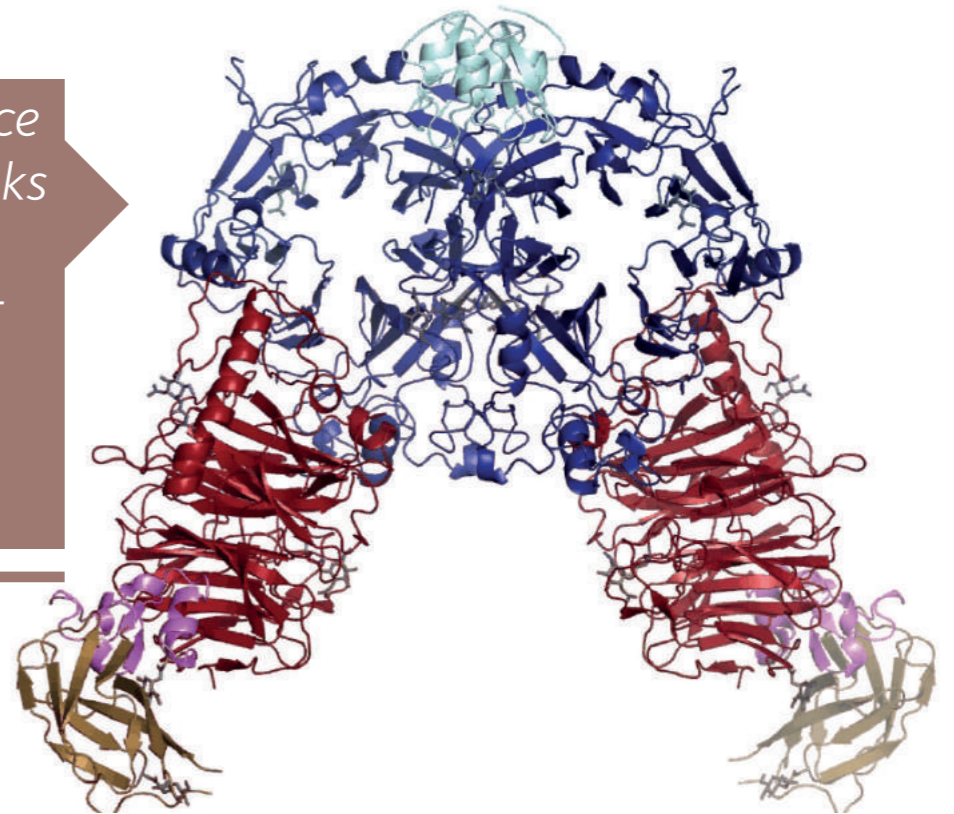
Development of mammalian expression systems for structural biology



Repulsive Guidance Molecules (RGMs) are fundamental in cell motility and adhesion, immune cell regulation and systemic iron metabolism. The crystal structure of RGM in complex with its receptor NEO1 allowed us to propose a mechanism for signal transduction at the membrane, where two RGM molecules conformationally stabilize the juxtamembrane regions of two NEO1 receptors, in a pH-dependent manner (Bell et al (2013) Science 341(6141):77–80)

First three-dimensional structure of a type-A γ -aminobutyric acid receptor (GABAAR). These receptors are the principal mediators of rapid inhibitory synaptic transmission in the human brain. The structure explains mechanistic consequences of multiple human disease mutations and reveals the binding mode of a previously unknown agonist, benzamidine. This opens a new avenue for the rational design of GABAAR modulators

The interaction of a cell guidance cue, semaphorin (blue), crosslinks two copies of a cell surface receptor, plexin (red), to trigger signals that contribute to the correct wiring up of the brain and central nervous system



WHAT WAS KNOWN

- Most proteins for structural biology studies are produced by high-level expression in a micro organism, Escherichia coli
- This approach fails to generate many human proteins, for example cell surface receptors
- Cell surface receptors provide the means of communication between cells in multi cellular organisms
- These receptors require special features (eg disulphide bridges to stabilize their structure and sugars linked to their surface) that cannot be produced in Escherichia coli

WHAT WE DID

- Developed a time- and cost-efficient system for high-level protein production in mammalian cells

WHAT THIS ADDS

- Production of proteins of biomedical interest (eg cell surface receptors) in a quantity and of a quality suitable for structural biology methods such as X-ray crystallography
- Many laboratories worldwide have used our methods to generate samples for structure determinations of proteins which were otherwise unobtainable, in particular for studies of cell surface receptors and secreted proteins.
- Within the WTCHG we are able to routinely produce proteins for cutting edge structure-function studies
- Numerous high impact analyses of cell surface receptor signalling mechanisms important to human health have been made possible

REFERENCES

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