# Solid-state NMR, synchrotron CD and oriented CD of a receptor tyrosine kinase transmembrane segment and its interactions with a viral oncoprotein

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## Protein-protein interactions inside the membrane

Membrane proteins and polypeptides are key players in many biological processes, as they control the flow of information and material between cells and their environment, and they are prime drug targets. The focus of our group lies on the structure-function analysis of membrane-active peptides and transmembrane protein segments of cell surface receptors. We are especially interested to find out how they insert into the membrane, how they fold and align within the hydrophobic core of the bilayer, and how they find and bind to their corresponding interaction partners. Complementary synchrotron circular dichroism (SRCD), oriented circular dichroism (OCD) and 2D solid-state PISEMA NMR measurements on macroscopically aligned samples are used to determine the conformation, alignment and dynamics of membrane-bound peptides and proteins in the quasi-native environment of a lipid bilayer. Here, we will present two interacting proteins: the PDGF-receptor β (PDGFR), a receptor tyrosine kinase, that gets activated by the oncogenic E5 protein via transmembrane helix-helix interactions.



*PDGFR dimer crosslinked by PDGF*    *PDGFR dimer crosslinked by E5*
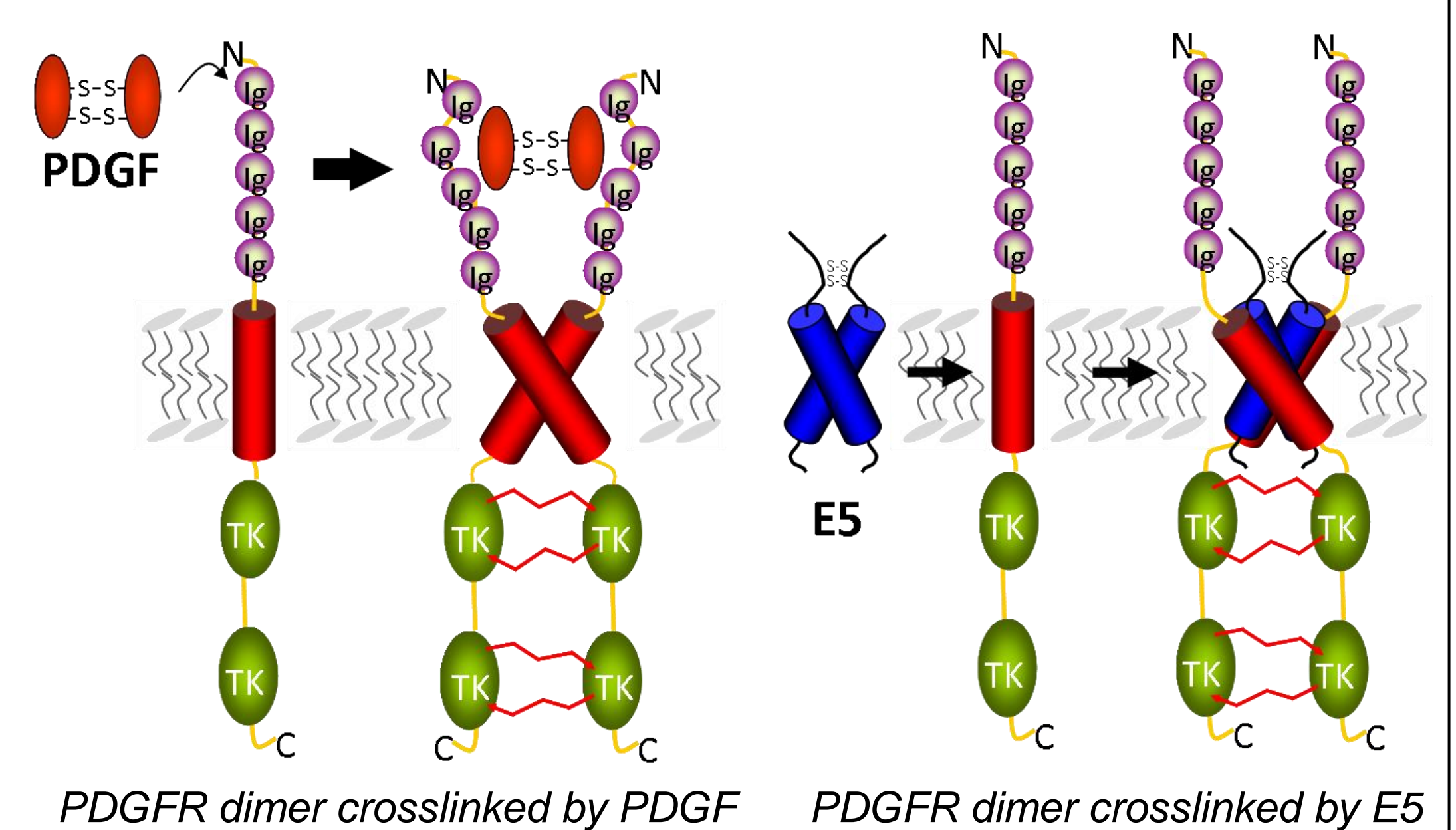
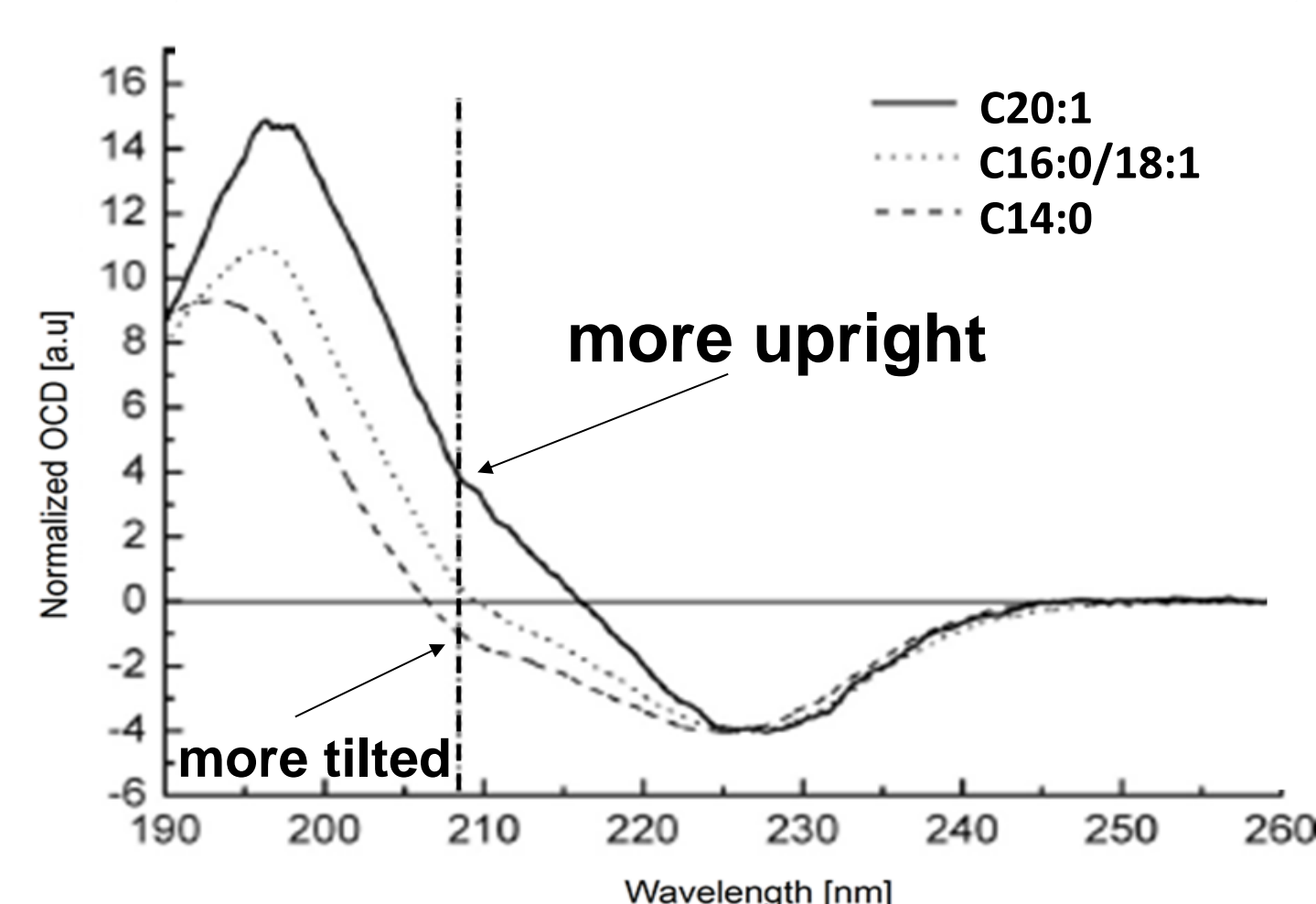## Complementary methods used in this study

### Synchrotron CD

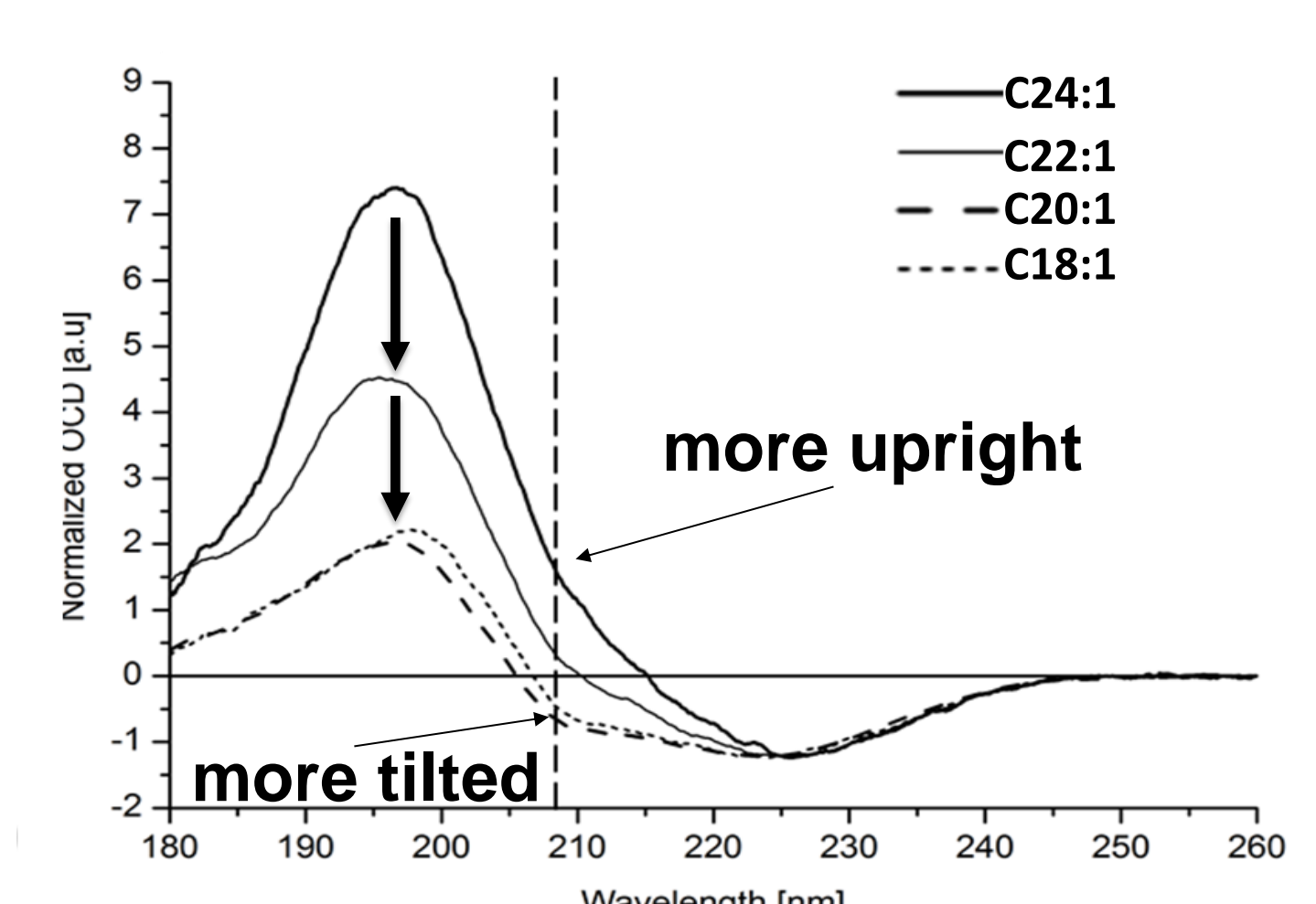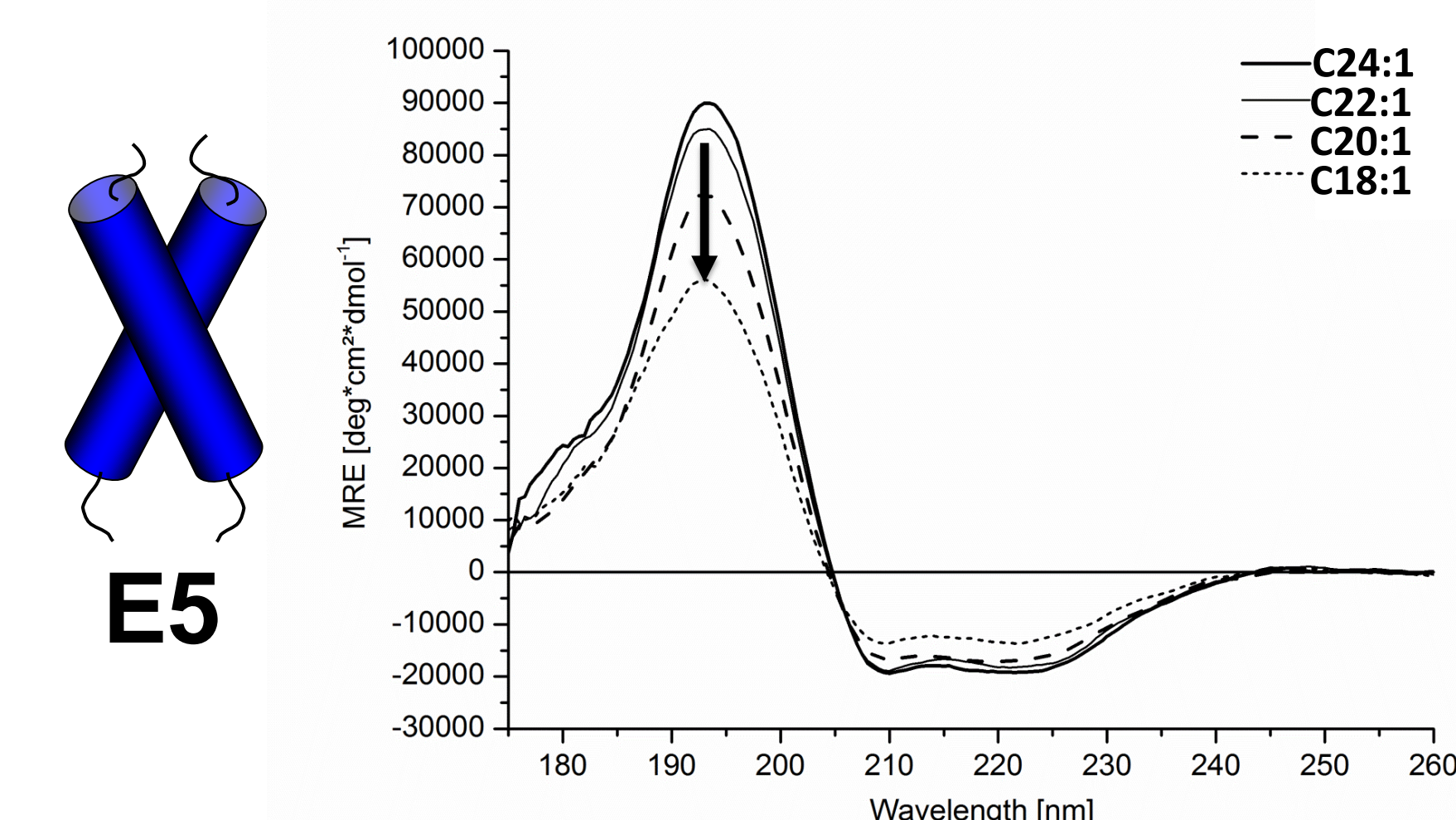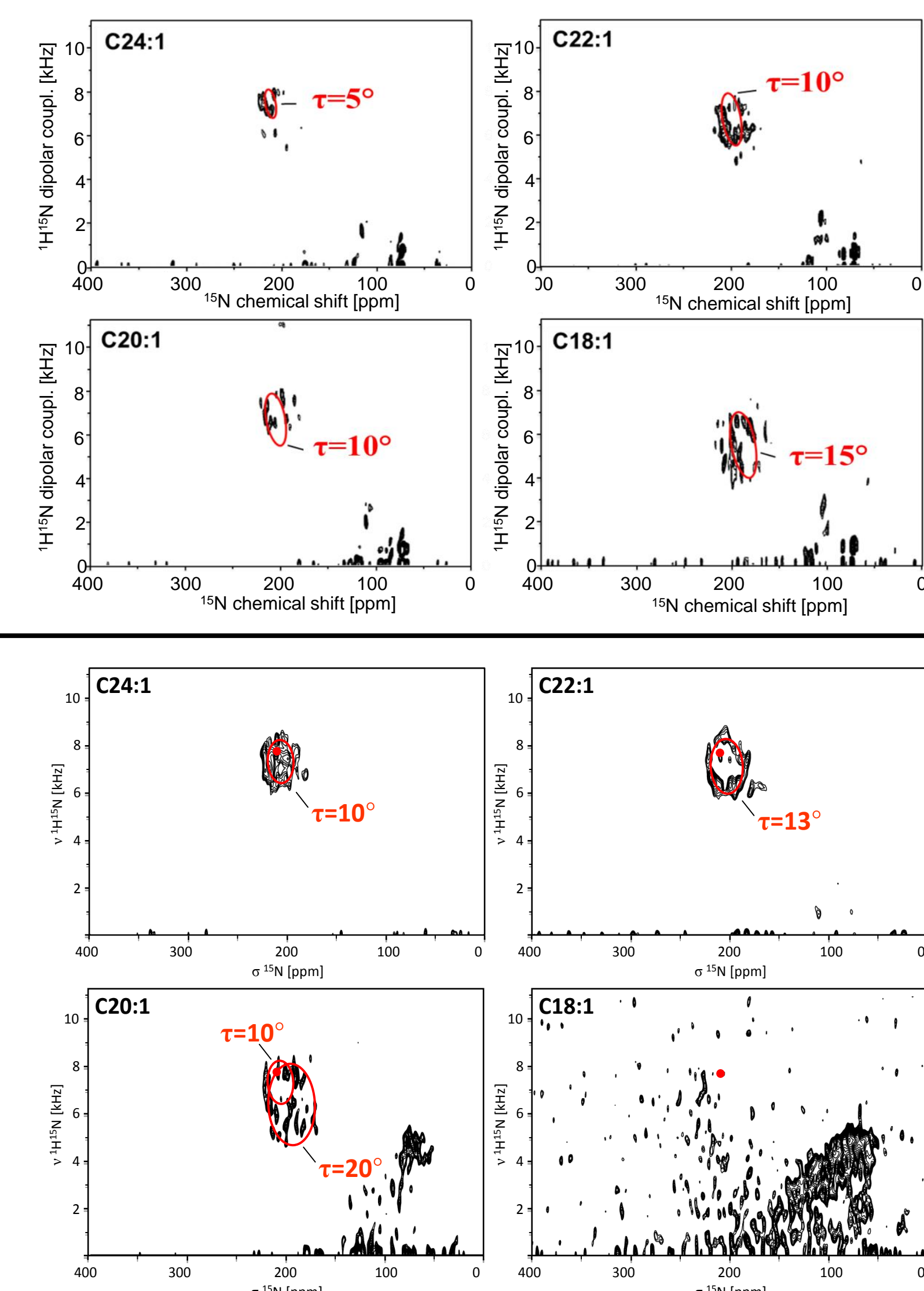*secondary structure and reconstitution*
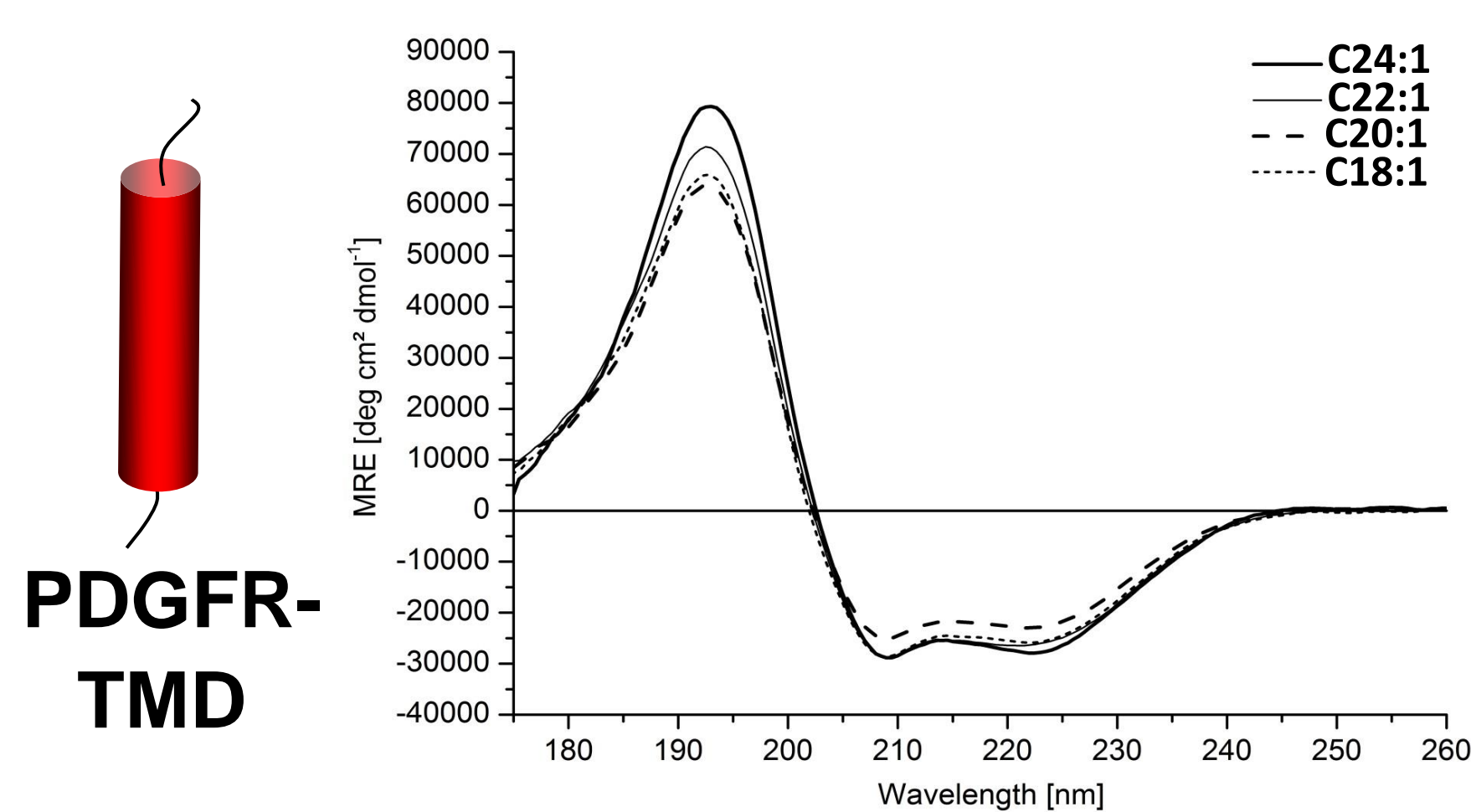
### Oriented CD

*orientation in membranes*

### PISEMA NMR

*helix tilt angle in membranes*



Analysis of the secondary structure and membrane alignment of E5 and the TMD of PDGFR. The proteins were reconstituted in lipid bilayers of different thickness and the influence on reconstitution, structure, tilt angle (with respect to the bilayer normal) and mobility was investigated by synchrotron CD, oriented CD and solid-state PISEMA NMR. By **SRCD** measurements a mostly α-helical secondary structure was found for both proteins. **OCD** measurements performed in lipids revealed a bilayer thickness dependent alignment of E5 and PDGFR: in thick lipid bilayers both proteins were upright inserted (positive 208 nm OCD bands), while with decreasing bilayer thickness (C24->C18) the helices were more tilted (negative OCD bands). **Solid-state 2D PISEMA NMR** measurements were used for a more detailed analysis of the tilt angles of both proteins. The tilt angles can be obtained by fitting simulated PISA wheels with defined tilt angles (red circles) to the measured spectra (black signals around 180-220 ppm). As it was also found by OCD, with decreasing bilayer thickness the helix tilt angles increased. In conclusion, the helices adapted a more tilted orientation to compensate the increasing mismatch between the hydrophobic length of the proteins and the thickness of the lipid bilayer. Moreover, for the E5 protein aggregation was observed when the membrane was too thin: reduction of the CD and OCD bands from thick to thin bilayer (arrows) caused by absorption flattening and light scattering of the aggregates and the loss of the PISA wheel in C18:1 lipid bilayers.

## E5 can interact with PDGFR through equally aligned transmembrane helices

When present in the same lipid bilayer, the E5 oncoprotein and the TMD of PDGFR share a similar α-helical secondary structure and a similiar orientational behaviour. This should allow a direct interaction of both proteins **through equally aligned transmembrane helices** and a close helix packing in the heterotetrameric bundle, resulting in sustained receptor activation.

The next step is the structural characterization of the **heterotetrameric E5/PDGFR-complex** to investigate which structural and orientational changes are induced by the complex formation.



E5    PDGFR    complex
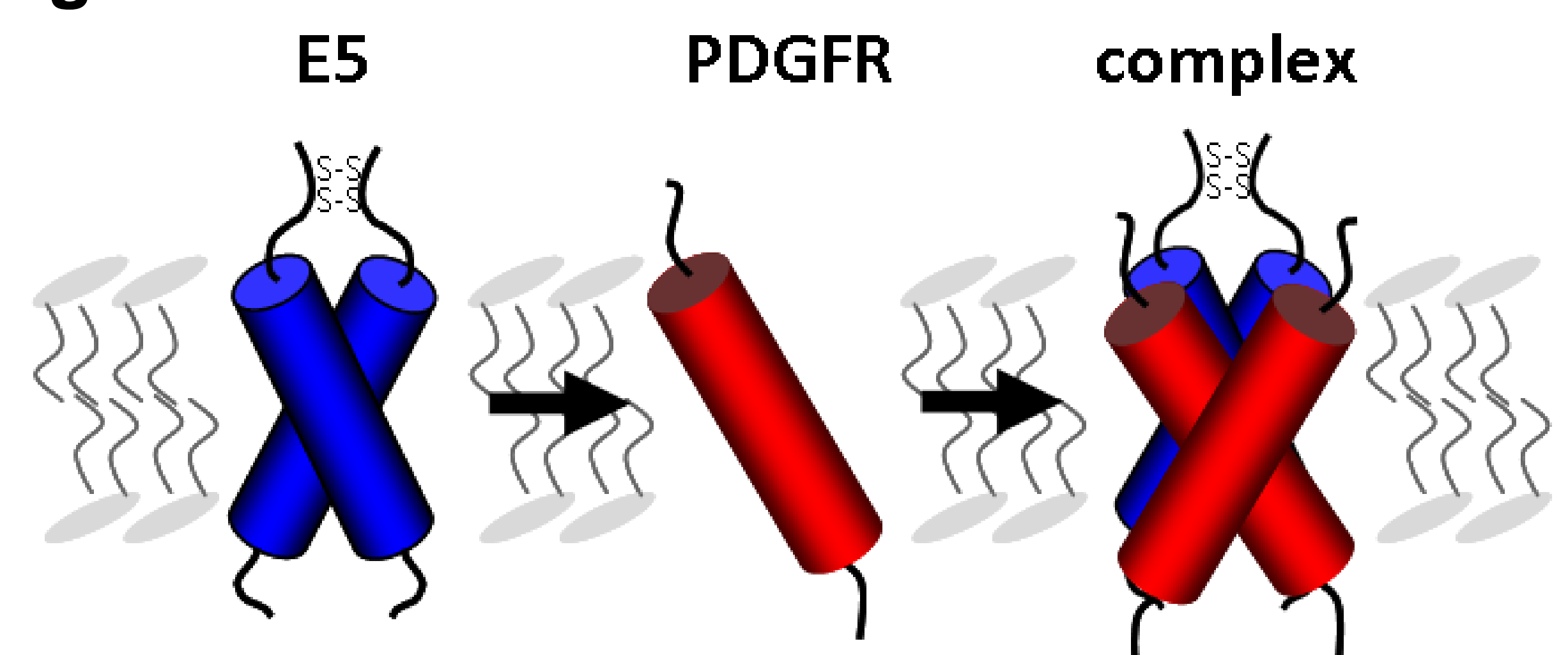
References for E5 and PDGFR:
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