Studying multi-site kinetics of Cadherin-11 dimerization by pressure variation and by chemical exchange detection

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Classical type I and type II Cadherins are calcium-dependent cell-cell adhesion proteins found in vertebrates. Classical cadherins consist of an intracellular domain, a single membrane-spanning alpha helix, and five extracellular domains EC1-EC5. The EC1 domains of Cadherins on apposite cell surfaces dimerize by swapping beta-strands as part of the adhesion process. In the EC1 dimer, the strand-swapping region of the beta-sheet includes anchor residues Trp2 in all classical Cadherins and in addition Trp4 in type II Cadherins. Solution NMR experiments with single domain EC1 constructs of Cadherin 8 (type II) have revealed the swapping strand is in exchange between an open (strand-exposed) and a closed conformation; it was also suggested that dimerization proceeds via a selected-fit mechanism [1]. However, the role of strand-exposed intermediates in type II Cadherins is still elusive [2,3] because of alternative dimerization routes in N-domain (N>1) constructs: Dimerization of E-Cadherin (type I) was shown to be able to proceed via an X-shaped dimer intermediate [4]. The intermolecular contact in X-dimer intermediates occurs in the EC1-EC2 linker region. The current main target of our Cadherin studies is Cadherin-11 (type II). Due to the very low K_D of Cadherin-11, observation of minor or major monomer states by NMR is challenging. We use one- and two-domain constructs of this protein and subject it to high-pressure NMR pressure spectroscopy to characterize kinetic multi-site exchange processes.

High pressure experiments can be used to unfold proteins, dissociate dimers and, more generally, increase the population of states with smaller partial volumes. We recorded and assigned spectra at varying concentrations, pressures, temperatures and pH for a Cadherin-11 EC1 construct. $^{1}H,^{15}N$ HSQC spectra show a number of different peaks for particular residues (Fig. 1). Relaxation data and information about chemical exchange, which is obtained from a customized NMR experiment, helped to deconvolute the information contained in the spectra. An unfolded state appears at higher pressures, in particular when the temperature is low: the protein is prone to cold denaturation at high pressure. Shifting from low to intermediate pressures, we observe that certain peaks, which do not belong to the unfolded state, gain intensity. These resonance peaks also are broadened by chemical exchange processes. We also have generated W2A and W2AW4A mutants which are thought to disrupt stand-swapping and promote flipping out of the β-strand. The obtained spectra resemble the WT spectra at higher pressures (Fig. 2). Pressure/temperature titration and exchange data for the W2A construct are different from the corresponding WT results and suggest that the observed peak patterns contain information about strand-swapping as well as the monomer-dimer equilibrium.

Spectra for the EC1 constructs overlay to a good extent with spectra for two-domain EC12 constructs. In addition to the insight into multisite kinetics of Cadherin-11 EC1 dimerization, we therefore also expect to be able to evaluate these processes in context of the two-domain construct.

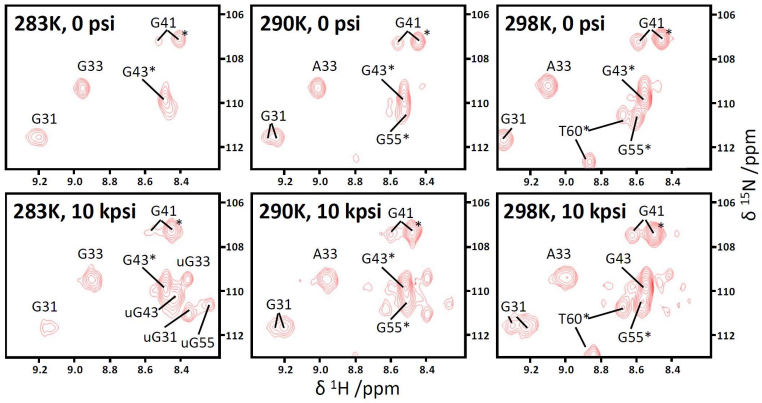

Figure 1: $^{1}H,^{15}N$-SOFAST HSQC spectra of Cadherin-EC1 at different pressures and temperatures. Conditions shown in figure. "u": unfolded. "*": higher intensity under pressure

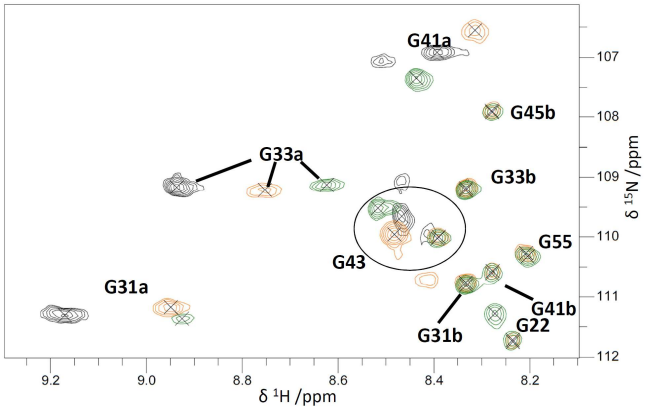

Figure 2: $^{1}H,^{15}N$-TROSY HSQC spectra of Cadherin-EC1 mutants. Black: WT; orange: W2A; green: W2AW4A. Spectra were recorded at 0 psi and 285 K.

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[3] Vendome, J. et al., *Nat Struct Mol Biol* **2011**, 18, 693-700.
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