Role of Conformational Heterogeneity in Domain Swapping and Adapter Function of the Cks Proteins*

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Cks proteins are adapter molecules that coordinate the assembly of multiprotein complexes. They share the ability to domain swap by exchanging a β-strand, β4. Here we use NMR spectroscopy and molecular dynamics simulations to investigate the dynamic properties of human Cks1 and its response on assembly with components of the SCF^{Skp2} ubiquitin ligation machinery. In the NMR experiment with the free form of Cks1, a subset of residues displayed elevated R2 values and the cross-peaks of neighboring residues were missing from the spectrum, indicating a substantial conformational exchange contribution on the microsecond to millisecond time scale. Strikingly the region of greatest conformational variability was the β4-strand that domain swaps to form the dimer. Binding of the ligand common to all Cks proteins, Cdk2, suppressed the conformational heterogeneity. This response was specific to Cdk2 binding; in contrast, binding of Skp2, a ligand unique to human Cks1, did not alter the dynamic behavior. Short time (<5 ns) molecular dynamics simulations indicate that residues of Cks1 that form the binding site for phosphorylated ligands are considerably more flexible in the free form of Cks1 than they are in the Cdk2-Cks1 complex. A cooperative interaction between Cdk2 and Cks1 is suggested, which reduces the configurational entropy of Cks1 and therefore facilitates phosphoryprotein binding. Indications of an unusual dynamic behavior of strand β4 in the free form of Cks1 were obtained from longer time scale (50 ns) dynamics simulations. A spontaneous reversible unzipping of hydrogen bonds between β4 and β2 was observed, suggesting an early intermediate structure for unfolding and/or domain swapping. We propose that the dynamic properties of the β-sheet and its modification upon ligand binding underlie the domain swapping ability and the adapter function of Cks proteins.

Cks1 is a member of the cyclin-dependent kinase subunit (Cks) family of proteins. Cks proteins were first identified as functioning in the control of the cell cycle by binding to cyclin-dependent kinase 2 (Cdk2) and regulating its function in some, as yet unknown, way (1). It is only recently, however, that their involvement in targeted protein degradation via the ubiquitin pathway has been discovered. First, in what is thought to be the canonical Cks function, it was found that they are required for the activating phosphorylation of the ubiquitin ligase APC (anaphase promoting complex) (2, 3); more recently Cks1, one of the two human Cks homologues, was shown to be essential for the activity of another ubiquitin ligase, SCF^{Skp2} (4–6).

The Cks proteins have very high sequence identity. The main difference is that some of the homologues have additional sequences at the N and C termini and a longer loop between the α-helices. The core structure is a four-stranded β-sheet. One face of the β-sheet forms the binding site for Cdk2, while on the other face there is a cluster of conserved residues that is thought to bind phosphorylated proteins (referred to as the phosphate-binding site) (7) (Fig. 1A). The less conserved α-helical region caps one end of the β-sheet and biochemical and mutagenesis studies have shown that this part of the protein binds the substrate recognition component, Skp2, of the SCF (6). Domain swapping has been observed for several Cks proteins with the C-terminal β-strand, β4, exchanging with another molecule to form a dimer pair (8–10). The process is mediated by a “hinge loop” sequence, and two conserved proline residues in the hinge loop are the main determinants of whether the protein adopts the monomer or the dimer form: mutation of the first or second proline creates an obligate monomer or dimer, respectively, whereas mutation of any other residue in the hinge loop has little effect on the monomer-dimer equilibrium (11).

Although the biological pathways through which Cks proteins act have been identified, the underlying mechanistic role re-
FIG. 1 A, structure of Cks1 showing the binding sites for Cdk2, Skp2, and phosphorylated substrate. The side chains of residues in the binding sites are shown, with residues in the Cdk2-binding site in green, in the phosphate-binding site in blue, and the Skp2-binding site in red. B–D, NMR spectra of Cks1 and Cks1 complexed with Cdk2 and Skp1-Skp2. B, HSQC of 15N-labeled free Cks1. C, TROSY-HSQC of 2H,15N-labeled Cks1 in complex with Cdk2 (red); 2H,15N-labeled free Cks1 (black) is shown for comparison. D, TROSY-HSQC of 2H,15N-labeled Cks1 in complex with Skp1-Skp2 (red); 2H,15N-labeled free Cks1 (black) is shown for comparison. Samples were prepared in 100 mM NaPO4 buffer, pH 6.8, 100 mM NaCl, 5 mM dithiothreitol, 5% D2O. The concentration of Cks1 was 150 μM, with Cdk2 or Skp-Skp2 in slight excess.
mains to be determined. They have been proposed to function as macromolecular adapters or docking factors. In the context of their canonical function, they are thought to facilitate or direct the binding of Cdk2 to a partly phosphorylated substrate such as APC by binding both Cdk2 and the phosphoprotein simultaneously. One requirement is therefore the presence of multiple binding sites. A second requirement may be the interdependence of these binding sites. A change in the conformation of the Cdk2-binding site altered the ligand affinity of the phosphate-binding site (12). Also, we have shown that human Cks1 directs the SCF<sup>Skp2</sup> ubiquitination machinery to its target p27 by binding Skp2, Cdk2, and p27 in a synergistic manner (13). To investigate the structural requirements of an adapter molecule, we have used NMR TROSY (transverse relaxation-optimized spectroscopy) (14, 15) and molecular dynamics simulations to probe the dynamic behavior of Cks1 and its response upon stepwise assembly with the components of the SCF<sup>Skp2</sup> machinery.

We show by NMR experiments that uncomplexed Cks1 is a conformationally heterogeneous molecule and that the most labile region maps to β4, the β-strand that undergoes domain swapping. Cks1 responds differently to binding different ligands. While the binding of Skp2 does not change the dynamic properties, the binding of the canonical Cks ligand, Cdk2, suppresses the heterogeneity. To gain more detailed information about the effect of Cdk2 binding on the flexibility of Cks1, we performed molecular dynamics simulations for free Cks1 and Cks1 in complex with Cdk2 using implicit (EEF1 (16)) and explicit water solvation. The experiments and simulations both show that the binding of Cdk2 restricts the conformational flexibility of Cks1 residues that comprise the phosphate-binding site, supporting a cooperative interaction between Cdk2 and Cks1 for the binding of phosphorylated ligands. Longer time scale dynamics (50 ns) were performed to provide insights into the unusual dynamic behavior of β4 detected by NMR. Cross-correlations observed in the simulations were used to examine coupling among the motion of different portions of Cks1 before and after Cdk2 binding. The results suggest that a dynamic response to ligand binding, i.e., a change in overall flexibility and a redistribution of conformations, contributes to the allostery (17, 18), rather than the more classical "enthalpic" mechanism of allostery induced by discrete structural changes.

**EXPERIMENTAL PROCEDURES**

**Protein Purification**

*Escherichia coli* expression plasmid for GST-Cdk2 was a generous gift of J. A. Endicott, University of Oxford, and GST-Skp1/Skp2 was a generous gift of A. Hershko, Technion, Haifa, Israel. Isotopes were obtained from Goss Scientific Instrument Ltd., Chelmsford, UK, and other chemicals were obtained from Sigma.

C41 or BL21DE3 cells were transformed with the previously described expression vector for Cks1 (19) and grown in 2 × TY medium (1.5% (w/v) tryptone, 1% (w/v) yeast extract, 0.5% (w/v) sodium chloride) containing ampicillin at 37 °C to an A<sub>600</sub> of 0.6. They were then harvested in sterile centrifuge bottles by centrifugation for 10 min at 5000 rpm at 4 °C. The cell pellet was resuspended in M9 medium in D<sub>2</sub>O with the appropriate carbon (glucose or [13C]glucose) and nitrogen sources (15NH<sub>4</sub>Cl), using twice the initial rich medium volume. Cells were grown for a further 2 h at 25 °C and induced with 0.1 mM isopropyl β-D-thiogalactoside overnight at 25 °C. Expression of Cdk2 and Skp1/Skp2 was carried out as described previously (13). Proteins were purified by nickel affinity (Cks1) or glutathione affinity methods (Cdk2, Skp1/Skp2) followed by gel filtration (Superdex 75, Amersham Biosciences). Proteins were judged to be >95% pure by SDS-PAGE. Mass spectrometry showed that Cks1 samples were >90% labeled with the appropriate isotopes. Samples were prepared for NMR in 100 mM NaPO<sub>4</sub> buffer, pH 6.8, 100 mM NaCl, 5 mM dithiothreitol, 5% D<sub>2</sub>O, and Complete protease inhibitor mixture (Roche Applied Science). The protein complexes were assembled with Cdk2 and/or Skp1/Skp2 in slight excess relative to Cks1. The complex was then dialyzed in the same beaker as a control sample of free Cks1, and NMR spectra were subsequently recorded in parallel. Samples were concentrated to ~150 mM using Vivatwin concentrators (VivaScience), then filtered and extensively degassed. To confirm that the Cks1 complexes were formed and remained assembled during NMR data acquisition, an aliquot of the NMR sample was loaded on a gel filtration column. An elution peak was observed at the volume expected for the respective complex. There were also minor peaks at volumes expected for Cdk2 and/or Skp1-Skp2 corresponding to the slight excess relative to Cks1 (used to ensure there was no free Cks1 in solution).

**NMR Spectroscopy**

Spectra were recorded at 298 K on Bruker DRX 600 and DRX 800 NMR spectrometers. Sequence-specific resonance assignments for 2H<sup>3</sup>C-labeled Cks1 wild type, both free and in complex with Cdk2, were made based on the following triple-resonance TROSY experiments: HN(CO)CA, HNCA, CBCA(CO)NH, HNCACB (as reviewed in Ref. 10). 15N-Edited three-dimensional NOESY experiments were acquired using 15N-labeled Cks1 to confirm and extend sequential assignments. For the resonance assignment of Cks1 in complex with Cdk2, the 1:1 complex of Cks1 was preformed with unlabeled Cdk2. Data were processed and analyzed with Felix 97 (Micron Separations).

TROSY-HSQC spectra of 2H<sup>3</sup>C-labeled Cks1 in complex with (unlabeled) Skp1-Skp2, with Cdk2, and with Skp1-Skp2 and Cdk2 were also recorded in 50 mM Tris-HCl, pH 7.5, 200 mM NaCl, 2% dithiothreitol, 5% D<sub>2</sub>O, and Complete protease inhibitor mixture (a buffer required for optimal stability of the Skp1-Skp2 component). The spectrum of free Cks1 was always recorded at the same time for comparison, using a sample that had been dialyzed in the same beaker as the sample of the complex.

Chemical shift changes upon Cdk2 binding were calculated as the root mean square deviation of the chemical shifts of corresponding peaks in free Cks1 compared with those in Cdk2-bound Cks1. The chemical shifts in the 15N dimension were divided by 10 to correct for the gyromagnetic ratio between H and 15N nuclei. Thus,

$$r.m.s.d. = \sqrt{\frac{(\delta_{\text{free}} - \delta_{\text{Cks1/Cdk2}})^2}{10^4}}$$

where δ<sub>free</sub> and δ<sub>Cks1/Cdk2</sub> are the chemical shift of residue i in free Cks1 in the proton/nitrogen dimension, and δ<sub>free/Cks1/Cdk2</sub> and δ<sub>Cks1/Cdk2/Cks1</sub> are the respective chemical shifts of residue i in Cks1 in complex with Cdk2.

**Relaxation Measurements**

15N T<sub>1</sub> and T<sub>2</sub> experiments were recorded at a 15N frequency of 60.13 MHz on a Bruker DRX600 spectrometer (as reviewed in Ref. 21). T<sub>1</sub> relaxation delays were set to 16, 80, 160, 240, 320, 400, 480, 600, 720, 840, 1000, and 1200 ms. T<sub>2</sub> relaxation delays were set to 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, and 240 ms. Peak volumes were fitted to a single exponential decay function using the program Kaleidograph (Abelbeck Software). In all the experiments a relaxation delay of 3 s was used. The two-dimensional experiments were acquired with 1024 × 128 complex points.

**Computational Methods**

**General Methods**

All calculations were carried out with the program CHARMM (22). For the calculations with implicit solvation the polar-hydrogen topology (PARAM19) with the EEF1 model was used (16), and for the simulations with explicit water the all-hydrogen topology (PARAM22) was employed (23). The crystal structure of the complex Cdk2-Cks1 (PDB entry 1BUH) served as starting point for the setup of all structural models.

**Calculations with Implicit Solvation Model**

**Setup of Structures**—The terminal residues 1–4 and 74–79 of Cks1 and 295–298 of Cdk2 are missing in the crystal structure and were neglected in the calculations, i.e., residue 5 of Cks1 was treated as the N terminus and residues 75 of Cks1 and 294 of Cdk2 as the C termini, respectively. Missing intermediate residues 40–46 of Cdk2 were constructed in the following way: initially placed coordinates were first minimized with 2000 steepest descent (SD) and 10,000 adopted basis Newton-Raphson (ABNR) steps. Subsequently, simulated annealing for these residues was performed by molecular dynamics, i.e., the residues were heated up within 20 ps to 2000 K and kept at this temperature for 500 ps and then cooled down to 50 K within 2 ns. The resulting struc-
ture was again minimized with 2000 SD and 5000 ABNR steps. The rest of the complex was kept fixed during this procedure. For the calculation of free Cks1 the Cdk2 unit was not present.

**Molecular Dynamics Simulation**—A trajectory of 50 ns was started from the minimized structure of free Cks1 using standard heating and early equilibration protocols (22) for the first 20 ps. The system was then coupled to a heat bath (300 K) applying the Berendsen algorithm. The leap-frog integrator was used with a time step of 1 fs (SHAKE constraints for all bonds of hydrogens to heavy atoms).

**Calculations with Explicit Water Solvation**

**Setup of the Structural Models**—To determine the protonation state of the ionizable groups, the corresponding pKₐ values for free Cks1 and the complex Cdk2-Cks1 were estimated from standard continuum electrostatics calculations (24). Residues His²⁰, His²¹, and His²⁹ of the Cks1 unit and Arg²⁰ of the Cdk2 unit were chosen to be ionized because they have pKₐ values higher than pH 7 in free Cks1 and the complex as well. These histidines were protonated; for all other ionizable residues their standard protonation state at pH 7 was used.

The N-terminal residue 4 of Cks1 was capped by an acetyl group and the C-terminus residue 74 by an alanine methyl group. For the simulation of Cdk2-bound Cks1, residues of Cdk2 further than 44 Å from the center of mass of the Cks1 unit were deleted; and for the simulation of Cks1 the entire Cdk2 unit was deleted. A sphere of pre-equilibrated modified TIP3P water molecules (radius 37 Å) centered on the center of mass of the Cks1 unit was overlaid, and water molecules overlapping with crystal structure elements were deleted. Crystal waters outside the overlaid sphere were deleted too. This resulted in an extensive solvent of the Cks1 unit with about six to eight water shells surrounding the protein. Bad contacts between the overlaid waters and the crystal structure were removed by performing 100 1000-step steepest descent minimization keeping protein atoms fixed. The water molecules were relaxed by a short dynamics run of 50 ps at 300 K (see below for details on MD). During this relaxation process, all protein atoms were kept fixed. This overlay and relaxation procedure was repeated five times with different orientations of the overlaying water sphere. This resulted in a total of 6905 TIP3P water molecules for free Cks1 and 6049 for Cdk2-bound Cks1, respectively. The outmost water molecules of the water sphere were deleted to obtain a density closest to that of pure water at 300 K and 1 atm (0.0334 molecules/Å³) for the volume occupied by the solvent. This resulted in 6769 waters for free Cks1 and 6147 waters for Cdk2-bound Cks1, respectively.

**Monizations and Molecular Dynamics**—Structures with equilibrated water molecules were minimized by 2000 SD and 5000 ABNR steps. For the minimization of the complex, Cdk2 residues more than 35 Å away from the center of mass of the Cks1 unit were kept fixed, and residues within 28–35 Å were confined to their original positions with harmonic restraints. The harmonic force constant for each residue was chosen proportional to the inverse of the average B-factor of this residue with a proportionality factor 4π²/735 (~7.9 kcal mol⁻¹ for T = 300 K), so that the fluctuations of the harmonic oscillations resembled the experimental fluctuations obtained from the B-factors of the x-ray structures. All other atoms were allowed to move freely. For the minimization of free Cks1, no restraints were used. MD trajectories of 5 ns were started from the minimized structures using stochastic boundary conditions with radius 37 Å (25). Water molecules within radius 30–37 Å were treated by Langevin dynamics with a friction coefficient of 82 ps⁻¹ for the water oxygen atoms and a heat bath temperature of 300 K. All other atoms were treated by Newtonian dynamics. The equation of motion was integrated with the leap-frog integrator using a time step of 2 fs (holonomic SHAKE constraints for all bonds of hydrogens to heavy atoms). All restraints of the minimizations were maintained. To keep the protein centered in the water sphere, the center of mass of the Cks1 unit was harmonically restrained using a force constant 50 kcal mol⁻¹ Å⁻².

**Quasiharmonic Analysis**—The Cα coordinates of the Cks1 unit were extracted from snapshots taken every 0.1 ps of the equilibrated MD trajectory (1–5 ns). The mass of each Cα atom was set to the total mass of the corresponding residue. The Cα structures were reoriented to remove translational and rotational modes. Then the mass-weighted fluctuation covariance matrix was diagonalized to obtain the quasiharmonic eigenvectors and frequencies (26).

**Cross-correlation Analysis of Atomic Fluctuations**—The Cks1 snapshot structures of the MD trajectory (1–5 ns) were first reoriented to remove translational and rotational modes before the normalized covariance matrix of atomic fluctuations was calculated. The backbone N plots in Fig. 10 only include cross-correlations between backbone N atoms. For the residue cross-correlation, the diagonal elements correspond to averages over all covariance elements involving atoms of the same residue, and the off-diagonal elements are obtained by averaging over all covariance elements involving atoms of two different residues.

**RESULTS**

**Experimental NMR Results**

**Missing Cross-peaks Indicate Conformational Exchange**

The HSQC spectrum of the 79-residue Cks1 is shown in Fig. 1B. It was immediately apparent that several cross-peaks were missing from the spectrum and that the cross-peaks observed had large intensity variation. This is indicative of conformational exchange on an intermediate NMR time scale. We screened a range of experimental conditions to eliminate alternative origins of line broadening. Spectra were recorded at protein concentrations from 40 to 1 mM, and no changes were observed in the line widths of the cross-peaks, indicating that aggregation was not involved. Furthermore, analytical ultracentrifugation and size-exclusion chromatography experiments indicate that the protein is >99% monomeric in this protein concentration range (data not shown). We attempted to recover non-observable cross-peaks by varying field strength (experiments were run on spectrometers with proton frequencies between 500 and 800 MHz) and temperature (273–303 K, well below the melting temperature of 328 K). Lowering the temperature to 278 K and increasing the field strength to 800 MHz resulted in additional very faint, broad peaks; however, the spectral quality was not good enough to obtain additional assignments. Experiments optimized for minimal solvent saturation did not recover additional cross-peaks, and this observation, as well as the lack of pH dependence, suggests that the absence of cross peaks for a subset of residues is not associated with rapidly solvent-exchanging amide protons.

We also looked at the mutant P62A, because the equivalent mutant (P90A) in Schizosaccharomyces pombe Cks protein Suc1 was found previously to greatly reduce the line broadening observed for that protein (27). In the case of Cks1, however, there was no significant difference between the spectra of P62A and wild type. Although the formation of a domain-swapped dimer is the other potential cause for the missing cross-peaks, the dissociation constant is too high for the dimer to be significantly populated (the dissociation constant for wild type Cks1 is in the 10 mM range (19), and it is at least an order of magnitude higher for the mutant P62A that showed similar line broadening). Moreover, the time scale of monomer-dimer interconversion is of the order of tens of minutes, much slower than the microsecond-millisecond time scale of conformational exchange that causes line broadening.

**Conformational Heterogeneity Maps to the Domain-swapping β-Strand 4**

The HSQC spectrum of Cks1 was assigned using standard triple resonance experiments complemented by NOESY-based techniques. To investigate the conformational exchange process further, the backbone dynamics was analyzed by measuring longitudinal (R₁) and transverse (R₂) relaxation rates (Fig. 2). The average ratio of R₂/R₁ of ~12 (corresponding to a correlation time t of 10.7 ns) is in the range expected for a 9 kDa protein. This is consistent with the results above that indicate the line broadening is not a result of Cks1 being oligomeric. A subset of residues display elevated R₂ values (Arg²⁰ in strand β, His²¹ and Glu²³ in the hinge loop between β3 and β4; these were excluded from the
calculation of the $R_2/R_1$ ratio, and surrounding these are residues without visible cross-peaks in the HSQC spectrum. These results strongly suggest that chemical exchange on a microsecond to millisecond time scale contributes significantly to the relaxation mechanism at these sites.

There was a single structural element for which there were no cross-peaks in the HSQC spectrum of Cks1. This stretch of sequence involves the domain-swapping β-strand 4. In addition to those residues adjacent to the missing cross-peaks, a number of other residues, mostly in the β-sheet, also exhibited significantly higher $R_2$ values than the average, notably residue Arg20 in the phosphate-binding site.

**Fig. 2. $R_1$ and $R_2$ values of free Cks1.** The secondary structure elements are shown above the plot.
Cks1 Dynamics Are Modified upon Binding of Cdk2

Cdk2 binds to one face of the Cks1 β-sheet (Fig. 1A) with an affinity of 1.5 μM (13). We looked at the effect of Cdk2 binding using 13C, 15N-labeled Cks1 in complex with unlabeled Cdk2. Due to solubility limitations, Cks1 was at a concentration of 150 μM with Cdk2 in slight excess. To improve the relaxation properties of the 44-kDa complex the side chain protons in Cks1 were perdeuterated (>80%) (28), and the spectral quality was improved by incorporation of TROSY building blocks (15). The TROSY-HSQC spectrum of Cks1 in the presence of Cdk2 is shown overlaid on that of free Cks1 (Fig. 1C). The two spectra were recorded in parallel on samples that had been dialyzed in the same beaker. The two spectra are very different. Most significantly, many more cross-peaks are observed from the Cks1 subunit as viewed from Cdk2 (C). Residues in dark blue are those that could be assigned in free Cks1, residues in orange and red are those that could be assigned only in the Cdk2-bound Cks1. Residues in orange are short extensions of sequence that could be assigned in the free form, whereas the residues in red, comprising β strand 4, are the only continuous stretch of sequence that is missing in the free Cks1 spectrum and that is recovered in the Cdk2-bound spectrum. Residues in yellow are those with elevated R2 values. D–F, chemical shift changes in Cks1 on binding Cdk2. Chemical shift changes upon Cdk2 binding were calculated as the root mean square deviation of the chemical shifts of cross-peaks in free Cks1 compared with corresponding peaks in Cdk2-bound Cks1. The chemical shifts in the 15N dimension were divided by 10 to correct for the gyromagnetic ratio between 1H and 15N nuclei. Thus, see Equation 1 under “Experimental Procedures.” Residues with a chemical shift change > 0.2 ppm are colored red on the structure of Cks1 and their side chains are shown. Residues with a change <0.2 ppm are colored blue. The greatest chemical shift changes are (i) Cdk2-binding interface: residues Asp14 and Glu15 (in the turn between β1 and β2) form ionic interactions with Cdk2 and are hydrogen bonded to Cdk2 backbone atoms; residues Tyr12 (β1) and His13 (β2) are hydrogen bonded in the complex with Cdk2. (ii) Distant from the Cdk2-binding interface: residues Arg20 and Asn45. These are key residues in the phosphate-binding site (Arg20) and the Skp2-binding site (Asn45). Different views of the protein are shown in A–C.

Cdk2 Binding Affects the Whole β-Sheet of Cks1

The large change in the spectrum required a reassignment of Cdk2-bound Cks1 using a series of triple resonance experiments and NOESY-based experiments acquired with a sample of side chain-perdeuterated 15N, 13C-labeled Cks1 in complex with unlabeled Cdk2. The changes on binding Cdk2 were then mapped onto the Cks1 structure (Fig. 3, A–C). Of the cross-peaks that could be assigned in the Cdk2-bound spectrum that were missing in the free Cks1 spectrum, most were 1-residue or 2-residue extensions of stretches of assignments that were made in the free form. The remaining additionally assigned residues mapped to a single stretch of sequence, the domain-swapping β-strand 4.

Changes in the HSQC spectrum of Cks1 on binding Cdk2 map to residues throughout the β-sheet as well as to residues in the α-helical region. First, the cross-peaks that were not visible in the free Cks1 spectrum due to line broadening were recovered upon binding Cdk2. Second, of those residues with cross-peaks that were visible in the free Cks1 spectrum many showed significant chemical shift changes (Fig. 3, D–F). Of significance are residues like Arg20 located in the phosphate-binding site on the opposite surface of the β-sheet to the Cdk2-binding site and Asn45 in helix α2 of the Skp2-binding site (Fig. 3, E and F).

Cks1 binds with high affinity to the 63-kDa heterodimer Skp1-Skp2, the substrate recognition component of the SCFSkp2 (13, 29). A sample of 150 μM 2H, 15N-labeled Cks1 was prepared in complex with (a slight excess of) unlabeled Skp1-Skp2 heterodimer. The 110-kDa complex of 2H, 15N-labeled...
Cks1 and unlabeled Cdk2 and Skp1-Skp2 was also assembled.

Analytical gel filtration of the NMR samples was used to confirm the formation of the complexes. The elution profiles showed one major peak at a volume expected for the complex and minor peaks corresponding to the non-Cks1 component(s), which were always used in excess of Cks1 to ensure there was no free Cks1 in solution. SDS-PAGE subsequently confirmed that all components of the complex were present in the major peak. No peak was observed at the volume expected for free Cks1, and no peak was observed at the void volume which would correspond to high order oligomers. Integration of the peak volumes showed that no sample was lost due to aggregation. Finally the correlation times for the Cks1-Skp1-Skp2 and Cks1-Cdk2-Skp1-Skp2 complexes were estimated based on the amide relaxation behavior and the values obtained agree well with those expected for their respective molecular weights.

Fig. 1D shows the TROSY-HSQC spectrum of Skp1-Skp2-bound Cks1 overlaid on that of free Cks1. The two spectra were recorded in parallel on samples that had been dialyzed in the same beaker. In contrast to Cdk2-bound Cks1 (Fig. 1C), the spectrum of Skp1-Skp2-bound Cks1 is similar in appearance to that of free Cks1, and notably no additional cross-peaks were recovered. There was an increase in line width, as would be expected due to the high molecular weight of the complex. There were also significant chemical shift differences compared with free Cks1, and these were mostly limited to residues in the α-helical region, Asp27, Glu36, Glu40, Glu42, Gln49, consistent with the interface mapped previously by site-directed mutagenesis (Fig. 1A) (6). Surprisingly Asn45, a residue found to be critical for the interaction with Skp2, does not show a significant chemical shift difference. The TROSY-HSQC spectrum of Cks1 in the quaternary complex of Cks1-Cdk2-Skp1-Skp2 was similar in appearance to that of the binary complex of Cks1-Cdk2. Thus, the NMR spectra indicate that the binding of Skp1-Skp2, unlike Cdk2, does not induce large changes in the internal dynamics of Cks1.

### Theoretical Results

**Flexibility of Native Ensemble Changes upon Cdk2 Binding**

The structures of Cks1 and its complex with Cdk2 have been solved by x-ray spectroscopy. There are two structures available for Cks1 (PDB entries 1DKS and 1DKT) (30). In both structures Cks1 is crystallized as a dimer; the structure 1DKS has a phosphate, and 1DKT a metavanadate, bound at the phosphate-binding site. In the x-ray structure of the Cdk2-Cks1 complex (PDB entry 1BUH) (7) the phosphate-binding site is unoccupied. The structure of Cks1 is very similar in all three crystals; the r.m.s. deviation of the Cα atoms is less than 1.5 Å (residues 5–74). Because of the higher experimental resolution, 2.6 Å (1BUH) versus 3.2/2.9 Å (1DKS/1DKT), we used the crystal structure of the complex as the starting point of the calculations for both the Cdk2-Cks1 complex and the uncomplexed Cks1. We simulated both systems for 5 ns with explicit solvation. The Cks1 unit was solvated in a sphere of water molecules (radius 37 Å), and the solvent molecules were confined to a sphere by using stochastic boundary conditions (25). For the simulation of complexed Cks1, the Cdk2 unit was modeled as illustrated in Fig. 4.

The simulations of free and Cdk2-bound Cks1 yield stable trajectories (Fig. 5A). The r.m.s. deviation curves for free and complexed Cks1 plateau after 1 ns of equilibration at approximately the same value of 1.3 Å. The average structures of the Cks1 unit calculated for the time interval 1–5 ns show Cα deviations from the complex crystal structure of 1.02 Å in the case of free Cks1 and 0.94 Å in the case of the complex. The main difference between these two structures is found in the...
bulge region of strand β1 (Ser9, Asp10, Lys11). In the complexed Cks1 the side chain oxygen of Ser9 forms a hydrogen bond with the backbone amide proton of His21 (strand β2, see Fig. 6A), as seen in all three crystal structures. This hydrogen-bonded configuration is not stable in the free form of Cks1. In the absence of the Cdk2 unit, the side chain oxygen of Ser9 forms an intrastand hydrogen bond with the backbone amide proton of Asp10 resulting in the loss of an interstrand hydrogen bond between β1 and β2.

The increased flexibility of the hinge loop region in free Cks1 can be understood by looking at the x-ray structure of the complex. There are intermolecular hydrogen bonds between the backbone of His36 (Cks1) and the side chain of Lys237 (Cdk2) and between the side chain of His36 (Cks1) and the backbone of Leu174 (Cdk2). Furthermore there is a salt bridge between the side chains of Glu61 (Cks1) and Lys237 (Cdk2). In the free form of Cks1 these interactions are missing and the hinge becomes more flexible. For the bulge in strand β1 there are no direct interactions with the Cdk2 unit that could explain the lower flexibility in the complex in comparison to free Cks1. Instead, residues Tyr12 and His21 that interact with both the bulge and the Cdk2 unit seem to constrict the fluctuations of Asp10 and Lys11.

The isotropic r.m.s. fluctuations of the backbone nitrogen atoms calculated from the simulations show that the flexibilities of free and complexed Cks1 differ considerably at two sites (Fig. 7). The backbone nitrogen fluctuations of residues 10–15, and especially Lys11 (bulge in strand β1) and Glu15 (loop β1-β2), and the residues of the hinge loop are significantly higher in the free structure of Cks1 than in the complex.

Atomic fluctuations can also be estimated from the experimental B-factors of crystal structures.

\[ B = \frac{8 \pi^2}{3} \langle \Delta r_i^2 \rangle \]  
(Eq. 2)

The correlation between the calculated fluctuations and those obtained from B-factors is reasonable, the correlation
coefficient amounts to 0.61 for the complex and 0.54 for free Cks1, respectively. The smaller correlation for free Cks1 is the result of large deviations for residues of the bulge region and the coil between a1 and a2. The disagreement for the bulge region can be understood if one considers that in the crystal structures of uncomplexed Cks1 (PDB entries 1DKS and 1DKT) the protein is present as a dimer and the two Cks1 units interact with each other at strand β2 (Fig. 8), which most likely suppresses the high flexibility of the bulge region and of those residues located across on strand β2 (Arg20, His21).

The change in flexibility of the entire stretch Ser9–Val22 and the alteration of the interstrand hydrogen bond configuration at the bulge region is a possible explanation for the chemical shift differences observed upon complex formation for a large number of residues (e.g. Tyr12, Asp14, Glu15, Arg20, His21, and Val22). Furthermore, the dramatic decrease of flexibility for residue Lys11 upon complex formation is in accord with the reappearance of the corresponding peak in the TROSY spectra. The immobilization of Lys11 upon complex formation may play a role in the adapter function of Cks1. Lys11, together with Arg20, Trp54, and Arg71, form the phosphate-binding site as seen in the crystal structure 1DKS (Fig. 9A). All four residues exhibit a higher flexibility in the free form than in the complex (Fig. 9, B and C), and therefore, the entropy cost of binding phosphorylated ligands should be smaller in the complex.

Cdk2 Binding Decreases Configurational Entropy of Cks1

The higher flexibility of free Cks1 in comparison with Cdk2-bound Cks1 can be quantified in terms of the vibrational entropy. We performed a quasiharmonic analysis (26, 31) for the MD simulations of free and complexed Cks1 using only the Cα atoms of the Cks1 unit. The mass of each Cα atom was set to the total mass of the corresponding residue. With the resulting normal modes, the vibrational entropy is as follows,

\[
S_{\text{vib}} = \sum_{k=1}^{N_{\text{vib}}} \left[ -N_A k_B \ln(1 - e^{-\Delta \omega_k \Delta T}) + \frac{N_A k_B v_k}{T} e^{-\Delta \omega_k \Delta T} \right] \tag{3}
\]

where \(N_A\) is Avogadro’s number, \(k_B\) is Boltzmann’s constant, \(h\) is Planck’s constant, and \(v_k\) is the frequency of normal mode \(k\). The summation is over all vibrational modes. The calculated vibrational entropy amounts to 917.3 cal mol\(^{-1}\) K\(^{-1}\) for complexed Cks1 and 943.1 cal mol\(^{-1}\) K\(^{-1}\) for free Cks1, respectively. Accordingly, the vibrational entropy differs by 25.8 cal mol\(^{-1}\) K\(^{-1}\), which corresponds to an energy difference of more than 7.5 kcal mol\(^{-1}\) at room temperature. If the decreased flexibility of Cks1 as seen in the complex with Cdk2 is necessary for the binding of a phosphorylated protein, the free form of Cks1 would need to overcome an additional entropic cost that could be as large as 7.5 kcal mol\(^{-1}\) for Cks1 to bind phospho-
rulyated proteins relative to that required in the Cdk2-Cks1 complex. The affinities of free and Cdk2-bound Cks1 for phosphopeptides have been measured by fluorescence anisotropy titrations. A small phosphopeptide, however, would be more flexible than an intact phosphoprotein and would probably not rigidify Cks1 to the same extent; the effect of Cdk2 complex formation on the phosphopeptide binding ability of Cks1 may therefore be small. The presence of Cdk2 slightly increased the binding affinity of Cks1 for phosphopeptides derived from the APC. Cdk2 also increased the affinity of Cks1 for a p27 phosphopeptide, if Skp2 was also present (13); Skp2 may reduce the flexibility of the p27 phosphopeptide. There are no experimental data available regarding the effect of Cdk2 on the affinity of Cks1 for intact phosphopeptides.

**Correlated Motions in Cks1 Are Suppressed upon Complex Formation**

Further insights into how complex formation changes the internal motions of the Cks1 unit are obtained from the covariance matrices of the spatial MD atom displacements. In the free form of Cks1 (Fig. 10A) there are strong positive backbone nitrogen cross-correlations between stretches of residues 6–14 (β1) and 18–25 (β2), 18–25 (β2) and 65–74 (β4), 54–60 (β3) and 66–70 (β4), and a strong negative correlation between 40–45 (α2) and 70–74 (β4, C terminus). We also calculated the residue correlations by averaging the covariance elements over all atoms of each residue. The resulting correlation pattern is similar to that obtained from the backbone nitrogen correlations but considerably less intense. The correlations between the β-strands are not surprising, since they interact with each other and together form the β-sheet. Remarkable is the strong negative correlation between helix α2 and the end of strand β4 (the C terminus), two structural motifs that are separated by more than 15 Å. This correlated motion between residues of the binding sites for Skp2 (α2) and p27 (Arg70 of β4) could play a role in the adapter function of Cks1 in that it provides a communication mechanism between the two binding sites. Upon complex formation with Cdk2 the correlations in Cks1 become weaker in general (Fig. 10B), in accord with the smaller amplitude of the fluctuations. Complex formation suppresses the correlated motions of the β-sheet and between α2 and the C terminus.

**Reversible Separation of Strands β2 and β4 in Free Cks1**

With reference to the NMR measurements, the cross-peaks of residues 65–71 are missing in the TROSY-HSQC spectrum of free Cks1 and reappear upon complex formation, which points to an increased flexibility of β4 in the free form of Cks1. However, β4 exhibits very low fluctuations in the MD simulations, not only in the complex but in the free form of Cks1 also. Indications about the unusual dynamics behavior of β4 were obtained from longer time scale dynamics. We simulated free Cks1 for 50 ns using the implicit solvation model EEF1. The Cα r.m.s. deviation from the crystal structure is illustrated in Fig. 5B. The r.m.s. deviation plateaus at about 7 ns to a value of 2.3 Å. There is a slight but distinct increase of the all-atom r.m.s. deviation at 20 ns. It drops down again at about 30 ns. Inspection of the MD trajectory reveals that a conformational change occurs at the C terminus (end of strand β4) in the time period between 22 and 32 ns. Two hydrogen bonds between β2 and β4 are broken and the side chain of Arg70 now bridges the two strands (Fig. 6C). Arg70 thereby acts as a “scissor” by “cutting” the interstrand hydrogen bonds. In the time interval 35–50 ns the Arg70 side chain moves out again and the two hydrogen bonds between β2 and β4 are reformed, as in the crystal structure (Fig. 6D). The “unzipping” of the terminal hydrogen bonds between β4 and β2 is certainly crucial for the domain swapping of β4 or the unfolding of β4 to an extended (open) form. Molecular dynamics simulation of the unfolding pathway of Suc1 (32) showed that the interaction between β2 and β4 is stronger than any other interaction between structural motifs; it is retained even at 500 K. Whereas Suc1 has to unfold almost completely to exchange strand β4 (33), Cks1 follows a different pathway with the interaction between β2 and β4 broken at the beginning followed by the separation of β4 from β3 (34). The conformation with the inserted Arg70 side chain may be regarded as an early intermediate structure on the pathway of unfolding for β4-strand exchange. The complete separation of β4 is, of course, not observed in the limited time of the molecular dynamics simulation, but the calculations have demonstrated two factors that might play an important role in the domain swapping process: (a) the terminal hydrogen bonds of β4 with β2 can be broken by the insertion of the Arg70 side chain, and (b) the hinge loop exhibits a very high flexibility in the free form that should facilitate the separation of β4 from β3.

**Weak Interaction between Cdk2 and Cks1 in the Complex**

We also studied the complex Cdk2-Cks1 with the implicit solvation model EEF1 by performing a normal mode analysis. An extensive structure minimization was necessary (see “Experimental Procedures”) to determine a minimum energy structure with only positive eigenvalues in the normal mode analysis. The r.m.s. displacement of the Cα atoms from the crystal structure after minimization is 1.31 Å. As a result of the minimization a slight movement of the two units, Cdk2 and Cks1, relative to each other is observed. The normal mode analysis shows that the complex structure features a very flat potential energy surface, e.g. the frequency of the three lowest modes (after the overall translational and rotational modes have been removed) is lower than 1.7 cm\(^{-1}\). The eigenvectors of these modes describe global motions of the Cks1 unit on the surface of Cdk2. This suggests that the interaction between Cdk2 and Cks1 is weak and that, therefore, the translational and rotational modes of the Cks1 unit relative to Cdk2 are low frequency modes of the complex. Indeed, the experimentally measured affinity of Cks1 for Cdk2 is modest (micromolar range) (13).

**DISCUSSION**

A Model for the Adapter Function of Cks Proteins—There is increasing evidence for the key role played by Cks proteins in the ubiquitin-proteasome pathway (2–5, 35, 36). Their importance was most recently highlighted by a study linking the Cks proteins to the function of the proteasome (37). The results of this study suggest that Cks proteins are required to recruit the proteasome for a non-proteolytic (and possibly also proteolytic) role in the transcription of CDC20 whose gene product activates the APC ubiquitin ligase. A common function of Cks proteins is therefore emerging in directing the assembly of a variety of protein complexes. However, the underlying mechanism remains unresolved. In this study we used the human protein Cks1, which assembles SCF/Skp2, Cdk2, and p27 and thereby drives p27 ubiquitination and subsequent degradation by the proteasome. There are two conserved surfaces on the Cks β-sheet that are involved in molecular recognition (Fig. 1A). One surface binds Cdk2 and although the identities of the substrates for the other site (referred to here as the phosphate-binding site) are unknown to date, the evidence points to phosphorylated proteins (such as p27) located within larger assemblies involved in ubiquitination pathways.

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2 S. E. Kelly, F. Herzog, J. M. Peters, and L. S. Itzhaki, unpublished results.
The NMR experiments show an exchange phenomenon for some residues of the β-sheet in free Cks1 on a microsecond to millisecond time scale, indicating the presence of more than one conformation, and this dynamic property is modified upon binding of the canonical Cks partner, Cdk2 (Fig. 3, A–C). The β-sheet of the Cks proteins is unusual as neither faces pack against any other structural element. Instead, it is very solvent-exposed with a conserved binding site occupying each of its faces (Fig. 1A). The solvent accessible surface (probe radius = 1.4 Å) for the 32 residues of the β-sheet is about 1900 Å² for free Cks1 and 1500 Å² for Cdk2-bound Cks1, respectively. Interestingly, the fraction hydrophobic/hydrophilic residues for its faces (1.6) is much higher than for the entire protein (1.0). The “naked” β-sheet may have evolved to allow the Cks adapter to bind multiple substrates simultaneously and the high degree of solvent exposure could be a factor contributing to the conformational heterogeneity.

The crystal structure of Cks1 is well defined with a relatively uniform distribution of B-factors over the structure and the different crystal derivatives (PDB entries 1DKS and 1DKT) overlay with small r.m.s.d. Thus, there is no indication of the heterogeneity that is observed in solution. Furthermore, the crystal structures of uncomplexed Cks1 and Cdk2-bound Cks1 are highly superimposable (Cα r.m.s. deviation is <1.1 Å for residues 5–74) and have similar B-factors (see Fig. 7), in contrast to their very different solution properties. This strongly suggests that the internal dynamics of Cks1 are frozen out upon binding Cdk2; the effect is mimicked in the crystal of uncomplexed Cks1 where the protein forms a dimer (see Fig. 8). Molecular dynamics simulations performed on a short time scale (~5 ns) show that the internal dynamics of Cks1 does not alter dramatically upon complex formation in terms of the cross-correlation for the fluctuations of different residues (Fig. 10), but a subset of residues features significantly lower fluctuations in the complex (see below). Moreover longer time molecular dynamics shows the existence of an inherent flexibility in the uncomplexed Cks1.

Conformational heterogeneity has been observed in response regulators such as Spo0F and nitrogen regulatory protein C (38–41). The data point to a mechanism of allosteric activation (42, 43) whereby the unphosphorylated protein interconverts between two conformers, populating predominantly the inactive one, and phosphorylation activates the protein by shifting this pre-existing ensemble to the active conformation. We can extend this mechanism of activation to a different class of proteins, macromolecular adapters like Cks1, where it is ligand binding rather than phosphorylation that induces the population shift (44–46). In view of the proposed biological role of Cks proteins as coordinators of multiprotein assembly, we suggest the following model for Cks actions. The exposed β-sheet of the Cks protein samples more than one conformation in solution and as such binds phosphorylated substrates with low affinity. Cdk2 binding to the β-sheet locks it in a single relatively rigid conformation, one that is assembly competent with a high affinity for its target proteins. Experiment and simulation both point to this model. Residues that display conformational heterogeneity in free Cks1 and that have an altered chemical environment upon binding Cdk2 include those in the phosphate-binding site (Fig. 3, A–C). Arg20 has an elevated R₂ value, and its cross-peak is significantly shifted on binding Cdk2; the cross-peak for Lys11 is not visible in the free Cks1 spectrum and is recovered in the Cdk2-bound spectrum. In the simulations, the bulge region of strand β1 (Asp10, Lys11) exhibits a significantly higher flexibility in the free form of Cks1 than in the complex. The binding of Cdk2 stabilizes the bulge region and prevents the switching between different hydrogen bond configurations seen in the free form of Cks1. Furthermore, Arg20 in strand β2, located opposite of the bulge of strand β1, displays an increased flexibility in the free form of Cks1, as do neighboring residues Tyr19 and His21. These observations support the recently proposed mechanism of crosstalk between the Cdk2- and phosphoprotein-binding sites (13); the modulation of the flexibility of the β1 bulge region, a structural motif common to Cks proteins, seems to play a key role in the communication. Moreover, once Cks1 is “locked” in one conformation, the calculations suggest that the entropy loss on binding a phosphorylated protein could be decreased by as much as 7.5 kcal mol⁻¹ upon complex formation with Cdk2. The model is in accord with the observed enhancement in Cks1 affinity for a p27 phosphopeptide (13) and for APC-derived phosphopeptides on binding Cdk2. An activation mechanism such as the one proposed may help to ensure that the assembly processes coordinated by Cks adapters take place in the correct order.

Domain Swapping as a Side Effect of Function—The ability to adopt different oligomeric states by domain swapping is common to all five members of the Cks family that have been studied in solution to date (8–10, 19, 47, 48). The finding that the domain-swapping β-strand in both Cks1 and Suc1 has conformational heterogeneity suggests that this facilitates the swapping process. The microsecond fluctuations that are detected by NMR are not sufficient to release the β-strand to domain swap but they may reflect a larger scale motion occurring in the partly unfolded state from which domain swapping occurs (11). A 50-ns molecular dynamics simulation for the free form of Cks1 suggests that one contribution to the unbinding of the β strand arises from the insertion of the side chain of Arg460, which separates β2 from β2.

It is important to note that neither the domain swapping ability nor the conformational heterogeneity arise from an intrinsic instability of the Cks structure; the stability of different family members varies widely and can be relatively high (from 3 kcal mol⁻¹ at 25 °C for Cks1 (19) to 6.5 kcal mol⁻¹ for Cks1 from Saccharomyces cerevisiae and 7.5 kcal mol⁻¹ for Suc1 (48)). It is also not the case that the Cks proteins are partially unfolded molecules, as kinetic and equilibrium unfolding studies show that they behave as cooperatively folded structural units (19, 48).

We do not know whether Cks proteins domain swap in vivo and/or whether this process is functionally relevant, but in view of the high dissociation constant of the dimer, this seems unlikely. Rather, the ability to domain swap may be a side effect of the conformational heterogeneity that is required for its adapter function. Do other proteins that domain swap also display such behavior in solution? Of the few domain-swapping proteins that have been studied by NMR, YopH and Cyanovirin-N display regions of internal mobility on the micro/millisecond time scale (49, 50). And two other proteins that domain swap, ribonuclease A and Spo0A, can adopt multiple conformations in crystals (51, 52). For some of these proteins, the structural heterogeneity may be relevant to the function.

Controlling Assembly and Activity of Protein Complexes—The canonical Cks function is thought to be that of an adapter between Cdk2 and phosphorylated substrates, and the solution properties of Cks1 reflect this role. First, the relatively modest, micromolar affinity for Cdk2 may be important to prevent Cks1 from being permanently attached to Cdk2 and to allow competition for Cdk2 between Cks1 and the other human Cks proteins, Cks2. As Cks2 does not bind to Skp2, this commits Cdk2 complexes to different signaling pathways. Second, the dy-

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3 S. E. Kelly, F. Herzog, and J. M. Peters, unpublished results.
4 M. A. Seeliger and L. S. Itzhaki, unpublished results.
namic properties observed here by experiment and simulation show how the binding of Cdk2 to one face of the β-sheet could enable the tight binding of phosphorylated substrates such as p27 to the other face. Such a mechanism would explain the absolute requirement of Cks1 to bind to Cdk2 in order for the ubiquitination of p27 to occur (6).

We showed previously that the affinity of Cks1 for p27 is enhanced by assembly of Cks1 with Skp1-Skp2 (13). The interaction between Cks1 and Skp1-Skp2 contrasts with that between Cks1 and Cdk2. The affinity for Skp1-Skp2 is very high (13), and this is consistent with Cks1 and Skp1-Skp2 being permanently assembled, as is suggested by their parallel expression levels during the cell cycle (6). The mode of binding of Skp2 to Cks1 revealed by NMR is also different from that of Cdk2 to Cks1. Skp2 binds to the α-helical region; i.e., chemical shift differences are localized to these residues, and there is not the dramatic change in internal dynamics that occurs when Cdk2 binds. In the absence of a crystal structure it remains unresolved how Cks1 and Skp2 work together to bind p27 with high affinity. Possible models include: 1) Skp2 binding induces structural changes in Cks1, 2) Cks1 binding induces structural changes in Skp2, and 3) Cks1 and Skp2 form a bimolecular interface for binding p27. The NMR results showing no global change in Cks1 structure or dynamics upon binding Skp1-Skp2 now allows us to rule out model (1).

The evidence presented here strongly suggests that the dynamic properties of Cks1, as revealed by NMR experiments and molecular dynamics simulations, are functionally relevant. First, conformational heterogeneity has now been seen for two Cks proteins, Cks1 and Suc1; second, this property is altered by binding of the canonical ligand. Interestingly, Elongin C, a protein that acts as an adapter in another ubiquitin ligase, CVCVHL, behaves similarly (53). On complex formation with Cdk2, the flexibility is significantly decreased for those residues of Cks1 that form the binding site for the phosphorylated ligand. This suggests a cooperative interaction between Cdk2 and Cks1, which reduces the configurational entropy of Cks1 and therefore facilitates substrate binding. Our results also have wider implications for dynamic mechanisms underlying signaling networks and for the organization of protein networks (17, 45, 54–58). The dynamic behavior of Cks1, as revealed by NMR experiments and molecular dynamics simulations, are functionally relevant. The evidence presented here strongly suggests that the dynamic properties of Cks1, as revealed by NMR experiments and molecular dynamics simulations, are functionally relevant.