ACCOUNTS of chemical research

Article

pubs.acs.org/accounts

Signal Sensing and Transduction by Histidine Kinases as Unveiled through Studies on a Temperature Sensor

Luciano A. Abriata,[†,‡,⊥] Daniela Albanesi,[§,⊥] Matteo Dal Peraro,[*,†,‡] and Diego de Mendoza[*,§]

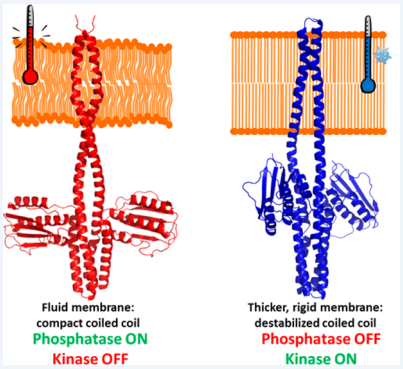
[†]Institute of Bioengineering, School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

[‡]Swiss Institute of Bioinformatics (SIB), CH-1015 Lausanne, Switzerland

[§]Laboratorio de Fisiología Microbiana, Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Ocampo y Esmeralda, Predio CONICET Rosario, 2000 Rosario, Argentina

Supporting Information

CONSPECTUS: Histidine kinases (HK) are the sensory proteins of two-component systems, responsible for a large fraction of bacterial responses to stimuli and environmental changes. Prototypical HKs are membrane-bound proteins that phosphorylate cognate response regulator proteins in the cytoplasm upon signal detection in the membrane or periplasm. HKs stand as potential drug targets but also constitute fascinating systems for studying proteins at work, specifically regarding the chemistry and mechanics of signal detection, transduction through the membrane, and regulation of catalytic outputs.



In this Account, we focus on *Bacillus subtilis* DesK, a membrane-bound HK part of a two-component system that maintains appropriate membrane fluidity at low growth temperatures. Unlike most HKs, DesK has no extracytoplasmic signal-sensing domains; instead, sensing is carried out by 10 transmembrane helices (coming from two protomers) arranged in an unknown structure. The fifth transmembrane helix from each protomer connects, without any of the intermediate domains found in other HKs, into the dimerization and histidine phosphotransfer (DHp) domain located in the cytoplasm, which is followed by the ATP-binding domains (ABD). Throughout the years, genetic, biochemical, structural, and computational studies on wild-type, mutant, and truncated versions of DesK allowed us to dissect several aspects of DesK's functioning, pushing forward a more general understanding of its own structure/function relationships as well as those of other HKs. We have shown that the sensing mechanism is rooted in temperature-dependent membrane properties, most likely a combination of thickness, fluidity, and water permeability, and we have proposed possible mechanisms by which DesK senses these properties and transduces the signals. X-ray structures and computational models have revealed structural features of TM and cytoplasmic regions in DesK's kinase- and phosphatase-competent states. Biochemical and genetic experiments and molecular simulations further showed that reversible formation of a two-helix coiled coil in the fifth TM segment and the N-terminus of the cytoplasmic domain is essential for the sensing and signal transduction mechanisms.

Together with other structural and functional works, the emerging picture suggests that diverse HKs possess distinct sensing and transduction mechanisms but share as rather general features (i) a symmetric phosphatase state and an asymmetric kinase state and (ii) similar functional outputs on the conserved DHp and ABD domains, achieved through different mechanisms that depend on the nature of the initial signal. We here advance (iii) an important role for TM prolines in transducing the initial signals to the cytoplasmic coiled coils, based on simulations of DesK's TM helices and our previous work on a related HK, PhoQ. Lastly, evidence for DesK, PhoQ, BvgS, and DctB HKs shows that (iv) overall catalytic output is tuned by a delicate balance between hydration potentials, coiled coil stability, and exposure of hydrophobic surface patches at their cytoplasmic coiled coils and at the N-terminal and C-terminal sides of their TM helices. This balance is so delicate that small perturbations, either physiological signals or induced by mutations, lead to large remodeling of the underlying conformational landscape achieving clear-cut changes in catalytic output, mirroring the required response speed of these systems for proper biological function.

INTRODUCTION

Organisms constantly sense and respond to extracellular signals in order to adapt to environmental changes and survive. For this, bacteria employ a large number of two-component systems (TCS), which consist of pairs of sensor and response regulator proteins specific to different stimuli.[1−3] The sensor protein of a typical TCS is a histidine kinase (HK), most often membrane-bound, that autophosphorylates upon stimulation and then transfers its phosphate group to its cognate response regulator.

Received: November 24, 2016

DOI: 10.1021/acs.accounts.6b00593
Acc. Chem. Res. XXXX, XXX, XXX−XXX

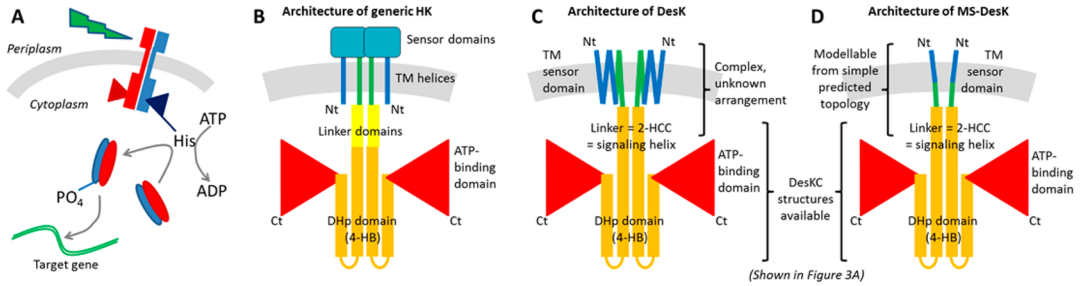


Figure 1. (A) A canonical two-component system featuring a membrane-bound histidine kinase and a soluble cognate response regulator. (B) General architecture of histidine kinases. (C) Architecture of *Bacillus subtilis* DesK, with no extracytoplasmic domains and simply a helical linker connecting the TM cold-sensing domain with the cytoplasmic domain (DesKC). (D) Architecture of MS-DesK, a functional minimal version of DesK. Nt = N-terminus and Ct = C-terminus.

The phosphorylated response regulator, a soluble protein, then alters the transcription of target genes whose activities counteract the source of the signal. Once the stimulus has vanished, the sensor protein switches back to its resting state to shut down the response (Figure 1A), often through the phosphatase activity of the HK itself.[4]

HKs of bacterial TCSs play roles in virulence and antimicrobial resistance responses, standing as potential drug targets given that they are absent from genomes of the animal kingdom, where phosphorylation signals are dominated by serine, threonine, and tyrosine kinases.[3,5−8] On top of this potential clinical relevance, TCSs are interesting systems for structural studies on signal transduction, specifically regarding how different sensor proteins transduce different signals into the same intracellular outputs, that is, activation of a kinase or phosphatase activity, within a regulatory circuit more complex than originally thought.[9]

The HKs of TCSs that respond to different stimuli share modular architectures with conserved "effector" domains with autokinase, phosphotransferase, and phosphatase activities toward specific cognate response regulators and variable "sensor" domains for specific signal sensing (Figure 1B).[3] Most HKs are membrane-bound homodimers with extracellular or transmembrane sensor domains poised to receive signals from the exterior and with effector and catalytic domains located in the cytoplasm,[10] where the soluble response regulator resides (Figure 1A,B). The sensor domain of HKs is N-terminal while the cytoplasmic effector domain is C-terminal and includes a central dimerization and histidine phosphotransfer (DHp) domain and the ATP-binding domains (ABDs). HK dimers are stabilized through strong interactions of two pairs of helices comprising a four-helix bundle (4-HB) that constitutes the DHp domain where the phosphorylatable histidine resides.[11,12] Of the two helices that each protomer contributes to the DHp, the N-terminal one is linked to the sensor domain through a long α-helix that corresponds to the "signaling helix" widely recurrent in proteins with sensor and effector domains as a 2-helix coiled coil (2-HCC),[13] often including also a small HAMP, PAS, or GAF linker domain (Figure 1B,C). Finally, membrane insertion is provided by one or more helices of a transmembrane (TM) domain, which connects the sensor domain to the signaling helix and HAMP/PAS/GAF domains.

Despite active research, it is not clear how the domains of histidine kinases act together to receive different signals from the environment and produce similar catalytic outputs. In particular, the complex architecture of histidine kinases has so far hampered the determination of structures for full transmembrane systems. Most research in the field has therefore advanced through biochemical functional studies on different mutants and chimeric constructs, which are interpreted in the light of partial X-ray structures and computational models and simulations of various kinds.[14] We focus in this Account on a membrane-bound histidine kinase called DesK, which acts as a temperature sensor in bacteria and has provided an excellent model system for structural and functional investigations on HKs through *in vivo*, *in vitro*, and *in silico* approaches. Whereas these studies aimed at elucidating the function of DesK itself, some of our findings help understand generalities of signal transduction by other HKs; likewise, we integrate knowledge on other HKs toward understanding DesK.

DesK-MEDIATED TEMPERATURE SENSING IN BACTERIA

Environmental temperature is a critical variable for microorganisms because it equilibrates quickly with their small volumes, modulating steeply the reaction rates and equilibria that underlie cellular biochemistry. In particular, the cytoplasmic membrane is strongly influenced by temperature because the phospholipid acyl chains undergo phase transitions in the range of temperatures that prevail in Earth's biosphere.[15] These phase transitions lead to steep dependencies of several membrane properties on temperature in the range from 0 to 40 °C and even in the narrower range from 25 to 37 °C, as we recently reviewed.[16]

DesK was discovered around 15 years ago by the de Mendoza group as the sensor protein of a TCS that maintains appropriate membrane fluidity at low growth temperatures in *Bacillus subtilis*.[17] DesK is encoded by the first gene of a small operon coding also for its cognate response regulator, DesR, and its only target is the *des* gene, which codes for a Δ5-acyl lipid desaturase.[18,19] Transcription from the P*des* promoter is triggered in *B. subtillis* when the temperature drops below ∼30 °C, inducing desaturase expression. Desaturase activity then introduces *cis*-double bonds into acyl chains that are attached to existing phospholipids, helping to restore appropriate membrane fluidity in the new environment.[17]

This Account summarizes our current understanding and hypotheses about DesK functioning. We analyze the structural features of DesKC in the two main functional states of the effector domain, discuss current proposals on how the primary cold signal is sensed by the TM region and transmitted to the effector domain through the TM and 2-HCC regions, and advance possible roles for unexplored conserved prolines of the TM domain.

DOI: 10.1021/acs.accounts.6b00593
Acc. Chem. Res. XXXX, XXX, XXX−XXX

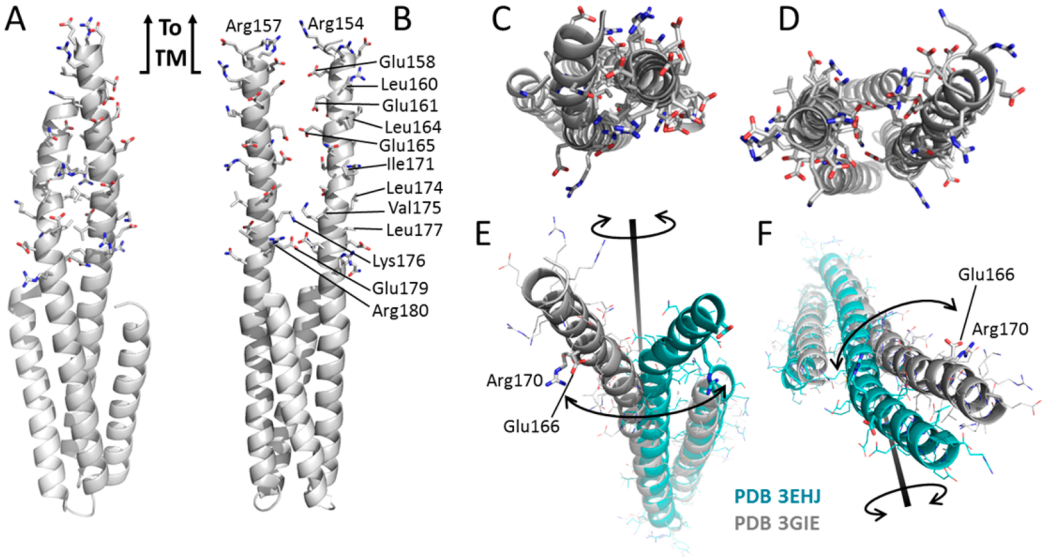


Figure 2. DesK's DHp domain and 2-HCC linker in the phosphatase-like state (A, C; PDB 3EHJ) or opened apart in a kinase-like state (B, D; PDB 3GIE). The backbone trace spans from the first observed residue (154−158 depending on PDB entry and chain) until Tyr210. Side chains of 2-HCC residues (until Arg185) are shown as sticks (red = oxygen, blue = nitrogen, gray = carbon). Some hydrophobic residues that become exposed and charged residues that become buried in the kinase state are labeled in panel B. Panels E and F compare chains A and B from both structures to highlight the 90° rotations of the helices as exemplified through Glu166 and Arg170. Interactive 3D views are available at https://lucianoabriata.altervista.org/papersdata/accounts2017.html.

GENERAL ARCHITECTURE OF DesK AND X-RAY STRUCTURES OF ITS CYTOSOLIC EFFECTOR DOMAIN IN DIFFERENT FUNCTIONAL STATES

DesK is a transmembrane HK lacking extracellular domains. Its TM region is composed of 10 TM helices, 5 coming from each protomer (TM1−5), arranged in an unknown fashion. TM5 connects directly into a signaling helix that ends up in the DHp domain (a 4-HB), without any HAMP, PAS, or GAF domains in-between (Figure 1C). Systematic deletion of DesK's TM segments showed that the TM topology can be simplified, retaining functionality. More precisely, a truncated minimal sensor version in which the first half of the first TM helix is fused to the second half of the fifth TM helix, dubbed MS-DesK, is fully functional both *in vitro* and *in vivo*[20] (Figure 1D). Full-length DesK and MS-DesK have been the subject of several structural and functional studies by our groups, shedding light on the mechanism underlying temperature sensing and putting forward some general ideas about HK functioning.

The first structural study on DesK reported nearly complete X-ray structures for its cytoplasmic portion (DesKC) in different functional states, including phosphatase- and kinase-competent states.[21] These structures highlighted a conformational change at the DHp 4-HB and the 2-HCC N-terminal to the DHp, central to the mechanism of activation in the kinase-competent form (Figures 1C,D and 2). Accommodation index analysis[22] of these structures reveals distortions of the 2-HCC that are relaxed over different lengths and through different asymmetries in each state (AI = −0.5 for the phosphatase state with maximum asymmetry at Arg180, while AI = +1 for the kinase state with peak asymmetry at Thr190, Figure S1), of potential functional significance.

Close comparison of DesKC structures hints at rotation and tilting of the two helices that enter the DHp from its N-terminal side (each helix coming from one protomer) as a route for signal transduction into DesKC (Figure 2). More precisely, each helix appears rotated by ∼90° around its long axis on its N-terminal end where the construct begins, a few residues after TM5. These rotations are such that the helices establish hydrophobic contacts in the phosphatase-competent state but not in the kinase-competent state, where some hydrophobic residues of the 2-HCC and the DHp 4-HB become exposed, that is, a destabilized conformation for the signaling helices (Figure 2).

TEMPERATURE SENSING RELIES ON MEMBRANE STATUS AS A PROXY

The genetic and biochemical studies[16,23] that led to the discovery of the Des pathway in *B. subtilis* suggested that DesK could assume different signaling states under varying growth temperatures.[17] Later, *in vivo* and *in vitro* studies on full-length DesK and truncated constructs of the TM domain suggested that DesK detects membrane thickness as a cue for cold sensing.[20,24] By increasing truncation of DesK's TM helices,[20] de Mendoza's group observed that TM1 and TM5 are both required for temperature-dependent regulation of the kinase output. They next observed that DesK constructs containing either TM1 or TM5 alone could not respond to the cold signal, whereas the chimeric TM1/5 construct MS-DesK responded to the cold signal similarly to full-length DesK.[20] Using this minimal construct, they further tested the effect of amino acid substitutions and insertions in the transmembrane portion of its helix, concluding that hydration, packing, and hydrophobic match to the membrane in MS-DesK's single TM helix tune functional output. In particular, the observation that altering the length of the hydrophobic segment of the TM1/5 chimeric helix alters functional output suggested that it acts as a ruler of membrane thickness.[20,24] Further experiments on MS-DesK with the wild-type sequence reconstituted in liposomes of different membrane thicknesses showed a more rapid and overall higher kinase output in liposomes made of lipids with longer acyl chains.[20] Moreover, *in vitro* and *in vivo* experiments

DOI: 10.1021/acs.accounts.6b00593
Acc. Chem. Res. XXXX, XXX, XXX−XXX

with full-length DesK also showed that lipids with longer acyl chains promote activation of the kinase activity of the sensor.[24,25] These results indicated that MS-DesK and DesK respond to membrane thickness by modulating its catalytic output. We note this does not imply that thickness itself is the true signal *in vivo*, as the increment in thickness of *B. subtilis* membrane phospholipids is ∼1−1.1 Å when going down from 37 to 25 °C,[26] i.e. smaller than the difference in thickness of the proteoliposomes where the activity of MS-DesK[20] and DesK[25] was stimulated *in vitro*. It is important to note that membrane properties other than thickness are affected by temperature and acyl chain lengths, including permeability to water and fluidity/rigidity, especially when fluid-to-gel phase transitions are caught in the relevant temperature range, hampering a clear assessment of which properties are actually sensed by the protein.[15,27−29] In any case, it is clear that DesK senses membrane status as a proxy for cold, through an unknown mechanism that further experiments and modeling studies attempted to unveil but that is presumably rooted in the internal mechanics of the TM bundle.

MECHANICS OF TRANSMEMBRANE HELICES AND THE SENSING MECHANISM

Subsequent work on MS-DesK proposed that a linker region connecting the TM helices' C-terminal ends to the helices that form the 2-HCC in the phosphatase-competent state of DesKC (and then enter the DHp) undergoes functional helix−random coil transitions upon temperature changes, providing the initial signal.[30] This hypothesis is interesting but is based on helix−random coil transitions experimentally observed for a small model peptide in solution, which might not reflect its true behavior in the context of an entire protein like MS-DesK or DesK. Moreover, it does not fit with any of the models proposed to date for HK functioning, which require motions of stiff elements to effectively transmit rotations, tilting or piston-like shifts of the helices into the DHp.[13,31,32]

Further work proposed that MS-DesK's TM helices rotate along their long axes to satisfy differential exposure of Ser143, Ser150, and Ser153 from a putative serine zipper located at the internal polar region of the membrane, generating the torque required to activate the DHp.[33] Although this mechanism fits better with the requirement of motions of rigid structural elements, it requires that the serines and other polar residues of MS-DesK's TM region be exposed to the hydrophobic membrane portion in the high-temperature phosphatase state, which is unlikely given the clear rules that govern the embedding of proteins in membranes.[34] We therefore do not favor the serine-zipper model for MS-DesK; however, we cannot rule it out for full-length DesK where the multiple helices could accommodate differential hydrogen bonding across TM helices in each state.

The situation of polar residues being exposed to the hydrophobic membrane region in the MS-DesK models from the latter study might be the consequence of using coarse-grained simulations, which can only account for rigid-body motions of different molecules with a limited description of conformational changes. In our latest work, we built atomistic (as opposed to coarse-grained) models of MS-DesK by extending structures of DesKC in the phosphatase- and kinase-competent states, to include the full chimeric TM1/5 helix.[35] For this we capitalized on the computational predictions that, after exiting the membrane, these helices continue all the way into the DHp and make a continuous 2-HCC with buried hydrophobic residues along all its cytoplasmic extension at least in the phosphatase state (Figure 3). Moreover, in the phosphatase-competent form the 2-HCC can be continued through the membrane toward the N-terminus with reversed polarity, that is with polar residues buried and hydrophobic residues exposed as expected for membrane-embedded proteins (Figure 3A). Due to the differences in the 2-HCC region of DesKC in the phosphatase and kinase states, satisfying the requirement of polar residues being buried and hydrogen-bonded across protomers in the TM region requires different orientations and conformations of the TM helices in each state (Figure 3B,C). They are tilted relative to each other continuing the coiled coil that comes from the cytoplasm in the phosphatase state, but they are nearly parallel and stretched in the kinase-competent state (Figure 3B). This different arrangement is accompanied by differential kinks of Pro148, close to the cytoplasmic exit of the TM helices. Furthermore, atomistic molecular dynamics (MD) simulations of these MS-DesK models in explicit solvent and membranes of different thickness revealed that the phosphatase state relaxes maintaining a symmetric structure and a stable 2-HCC similar to that of the starting model all the way through the membrane and the cytoplasm into the DHp, with distortions introduced locally at the two prolines of each helix (Pro16 and 148 in DesK numbering), whereas the kinase model drifts away from its starting conformation adopting strongly asymmetric conformations (Figure 3C).[35] Although we could not sample actual conversions between both states in our simulations, our observations led us to hypothesize that stretching of TM5 forces uncoiling of the 2-HCC in the TM region, building a torque that is then transmitted to DesKC's helices. Helix stretching to render the TM helices more parallel in the kinase form could be induced by changes in membrane thickness, stiffness, and hydration potentials.

SIGNALING INTO THE EFFECTOR DOMAIN IS CONTROLLED BY A TWO-HELIX COILED COIL

As described in the introduction to DesK's architecture, X-ray structures of DesKC suggest that the initial signal, originating at the membrane, should lead to opening and rotation of the helices along their long axes in the cytoplasm (Figure 2). As mentioned in the previous section, modeling of MS-DesK (and of TM5-DesKC[35]) suggests that DesK's TM5s run all the way into the DHp, making a continuous 2-HCC with buried hydrophobic residues along all its cytoplasmic extension in the phosphatase state (Figure 3). In the kinase-competent form, however, coiled coil packing in the cytoplasm side is not possible because the 90° rotation of both helices brings together the charged bulky side chains that point outward in the phosphatase state (Figures 2B,D,F and 3A). Therefore, the coiled coil is expected to be stable in the phosphatase-competent state but disrupted in the kinase-competent state, hinting at an essential functional feature that we and others have further explored. In fact, a role for 2-HCC stability in signal transmission in HK-like proteins was experimentally anticipated by Tao et al. for the yeast Sln1, the kinase of one of the few eukaryotic TCSs.[36]

We directly tested the role of 2-HCC stability on DesK's functioning by studying mutants aimed at stabilizing and destabilizing it.[35] In the destabilized mutant, hydrophobic residues of the cytosolic portion of the 2-HCC were replaced by hydrophilic residues (Ala167Arg, Ile171Gly, Leu174Gly); in the stabilized mutant, polar residues close to the exit of TM5

DOI: 10.1021/acs.accounts.6b00593
Acc. Chem. Res. XXXX, XXX, XXX−XXX

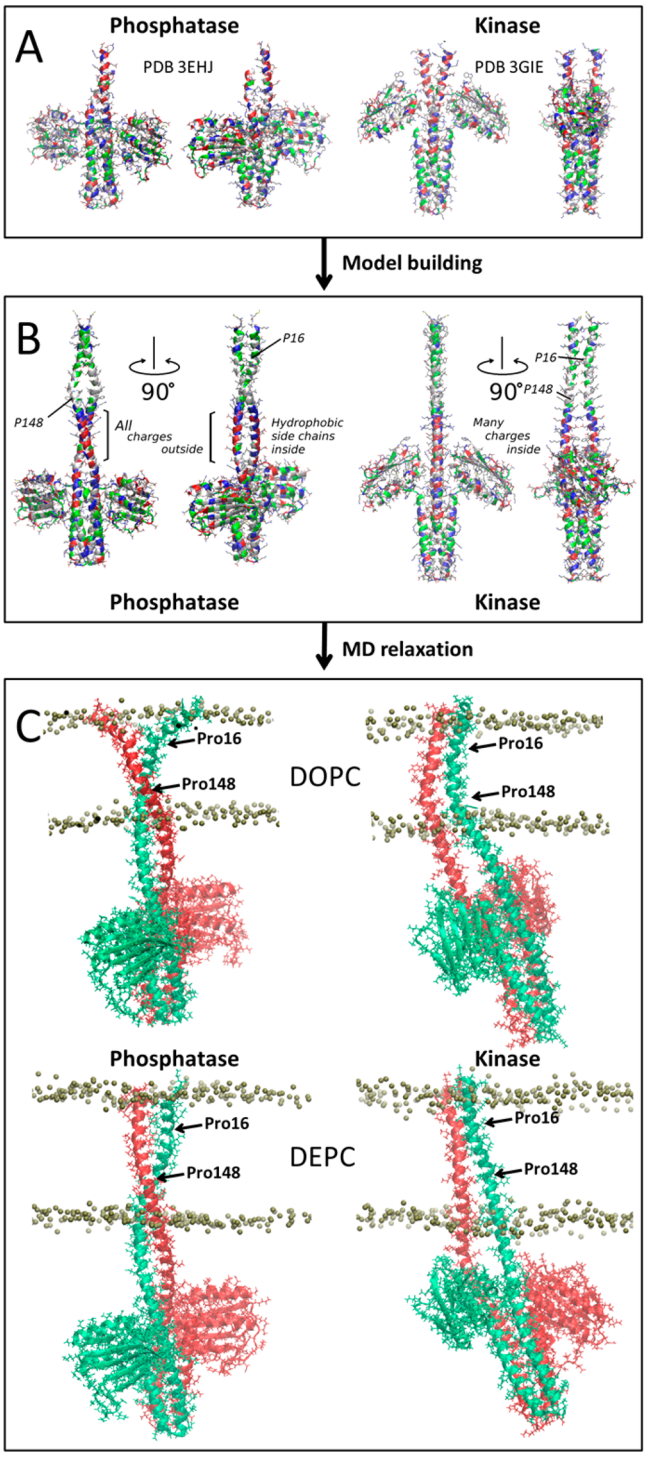


Figure 3. (A) X-ray structures of DesKC in phosphatase- and kinase-competent states. (B) Models of MS-DesK built by extending 2-HCC helices into TM5 based on bioinformatic predictions and considering that polar residues must be paired across monomers in the hydrophobic membrane portion (see ref 35). In panels A and B, residues are colored by polarity (red = negative, blue = positive, green = polar uncharged, gray = hydrophobic). Panel C shows how these two models relax after >100 ns of simulation at 303 K in DOPC (1,2-di(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine) and DEPC (1,2-dierucoyl-*sn*-glycero-3-phosphocholine) membranes, the latter being thicker and stiffer (tan spheres are P atoms from lipid phosphate groups).

from the membrane were replaced by hydrophobic residues (Ser150Ile, Ser153Leu, Arg157Ile). Atomistic models of the wild-type and stabilizing/destabilizing mutants built by extending the structure of DesKC in the phosphatase state to include full TM5 and relaxed through atomistic simulations supported our design of the mutations.[35] The destabilized mutant underwent rapid hydration of the 2-HCC around Arg167−Gly174 and Ala144−Arg157, the latter including Pro148 and comprising the last portion of TM5 before it exits the membrane. Both segments 167−174 and 144−157 remain compact in the stabilized mutant, and only 144−157 is hydrated in wild-type TM5. Experimental activity measurements clearly proved that the stabilized 2-HCC is associated with the phosphatase-competent conformation whereas loosened coiled coil packing favors the kinase-competent state.[35]

We thus concluded that switching of DesKC's catalytic output is determined by stabilization/destabilization of the 2-HCC, very likely through rotations along their long axes and through separations upon kinase activation. As described above, rotation of the helices upon moving from the phosphatase to the kinase form brings big charged amino acids inside the 2-HCC, consistent with helix separation and therefore destabilization en route to activating the kinase output. Moreover, Lesne et al.[37] showed that stabilization/destabilization of an analogous 2-HCC in the BgvS HK regulates its kinase/phosphatase output. Also in this system, mutations expected to compact the 2-HCC promote the phosphatase state, and mutations expected to loosen it promote the kinase state.[37] That work also showed that the phosphatase state is overall more stable while the kinase state is more dynamic,[37] in line with stronger distortions observed in our MD studies for the kinase state of MS-DesK (Figure 3C, right).

LINKING SIGNAL SENSING TO SIGNAL TRANSDUCTION: A ROLE FOR PROLINES?

It is clear that the main input into DesKC is a 90° rotation and separation of the signaling helices that make up the 2-HCC and connect into the DHp, driving destabilization of the 2-HCC. But what drives helical rotations upon sensing? While addressing this requires understanding the sensing mechanism itself, the key to the coupled sensing and transduction problem must lie in the mechanics of the TM helices. Toward a preliminary exploration of TM mechanics, we performed for this Account unbiased atomistic MD simulations of each of DesK's TM helices (TM5 actually extended into the 2-HCC) in membranes of two extreme fluidities but similar thickness (DOPC and DSPC, fluid and gel at 303 K, respectively). These results on single TM helices do not necessarily hold for DesK or even MS-DesK, but probe mechanical effects that often help understand this kind of system.[35,38−40]

From a single MD run on each system, the clearest observations are that (i) TM2, 3, and 4 adopt similar tilting angles in both membranes, while helices 1 and 5 can in principle adopt different angles (Figure S2) and (ii) fluctuations are reduced in the DSPC membrane for all TM helices. The latter observation indicates that membrane fluidity readily influences helix mobility, supporting our idea that changes in membrane properties other than thickness could generate the initial signal. The former observation in turn suggests that TM1 and TM5 could be the key sensor elements, in line with MS-DesK being functional.[20] The simulations further show kinking of the TM helices at proline residues, interestingly Pro16 and

DOI: 10.1021/acs.accounts.6b00593
Acc. Chem. Res. XXXX, XXX, XXX−XXX

Pro148, which locate internally (more than 2 helical turns away from the closest helix terminus). These two prolines are interesting because they are quite conserved (Figure S6) and correspond to the two only prolines in MS-DesK. Proline itself has interesting structural properties, introducing kinks and flexible hinges in helices, with potential for *cis*−*trans* isomerization, and this amino acid is abundant in fibrous proteins of large recoiling capacity.[41,42]

In five replicas of simulations of TM1 and TM5 in DOPC and DSPC membranes, we observe some differences across helices and membrane types (Figures 4, S4, and S5). The C-terminal half of TM1 tilts at ∼20° from the membrane normal oscillating by ±20° in the 5 DOPC replicas but instead gets restricted to different angles with smaller oscillations in each DSPC replica (Figure S4). Likewise, the N-terminal half of TM1 spans angles of 45−75° in DOPC with good overlap among replicas but is more restricted in DSPC, even getting close to 90° in two replicas. The differential tilting of each half of TM1 reflects a flexible hinge around Pro16, where the two helices swing, spanning angles of ∼80° (nearly perpendicular) to ∼160° (nearly parallel, Figure S5).

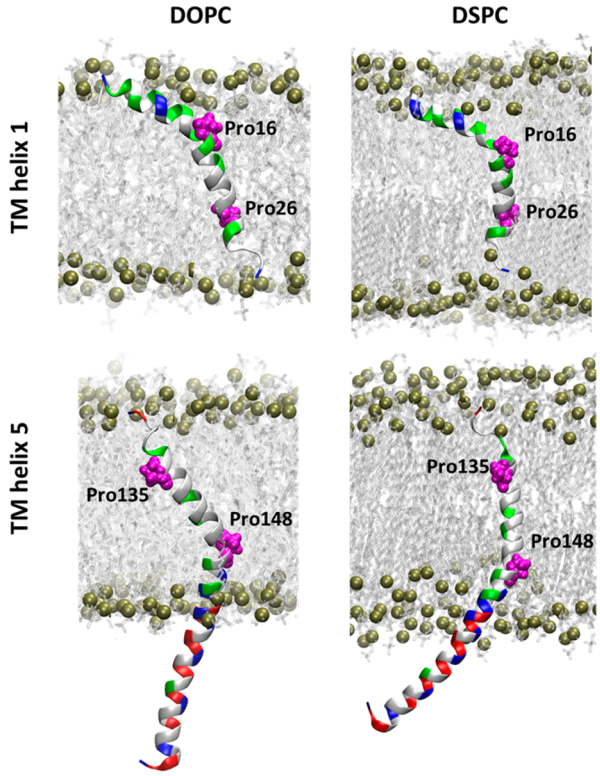


Figure 4. Orientation of DesK's TM1 and 5 continued into the cytoplasm after 300 ns of atomistic simulation at 303 K in DOPC and DSPC membranes (1,2-dioleoyl-*sn*-glycero-3-phosphocholine and 1,2-distearoyl-*sn*-glycero-3-phosphocholine, respectively). Figures retrieved from replica 1; see SI for data from 5 replicas and additional information. Prolines are shown in magenta; other colors as in Figure 3A.

Over 5 replicas for TM5 there are less clear differences between DOPC and DSPC, other than a rather constant relative tilting of the two helices such that the interhelical angle is rather constant along the trajectories and similar in the two membranes (135−145°, Figure S5). A clear difference thus arises between TM1 and TM5: in TM1, Pro16 acts as a hinge, likely facilitated by the conserved Gly13, located one helix turn upstream (Figure S6), such that the C-terminal half remains rather parallel to the membrane normal while the N-terminal half swings. In contrast, Pro148 in TM5 seems to create a rigid kink where both helix halves move together, exchanging between poses where one is parallel to the membrane and the other tilted.

These exploratory simulations and analysis, although not conclusive, clearly point at prolines as the subject of future research in DesK and possibly also other HKs. The dynamic hinge at Gly13−Pro16 was reported as thickness-sensitive in our computational study on MS-DesK, forcing hydrogen-bonding of the polar residues N-terminal to Pro16 (Gln9, Asn12, Tyr17) in thick membranes and their exposure to external solvent in thinner membranes.[35] Modulation of hydrogen bonds and hydration across TM helices was also reported for the TM domain of the PhoQ HK and proved functional.[39,43] Regarding the rigid Pro148 kink in TM5, while the details are certainly complex and yet not clear, we propose it acts as a fulcrum around which TM5 can pivot, producing scissoring motions that are translated, presumably as rotations, to subsequent domains (schematized for DesK in Figure 5).[44]

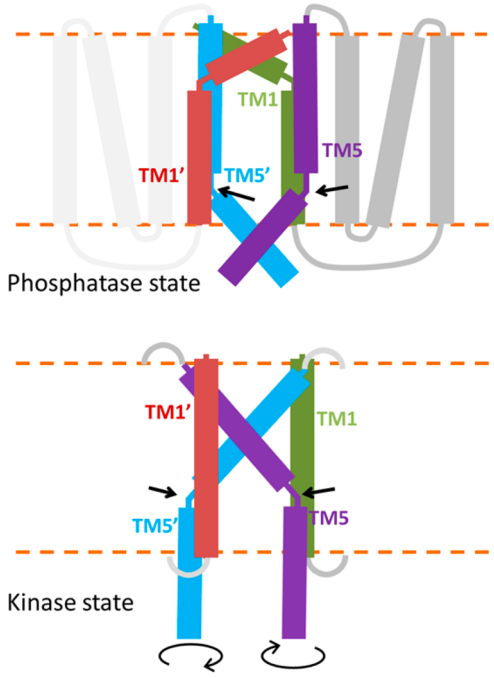


Figure 5. Scheme of how the Pro148-induced kink (black arrows) could guide rearrangements of helices 1 and 5 to drive the scissoring motion that shifts between compact and loosened cytoplasmic 2-HCC in DesK. In PhoQ, Pro208 generates a kink at a similar location in the membrane.[39] The closest structural representation of these states is given by the models of MS-DesK in Figure 3C. TM helices 2−4 are omitted in the model of the kinase state for simplicity.

This is similar to the role proposed for PhoQ's Pro208, which lies in a position similar to DesK's Pro148, that is, close to the phospholipid head groups of the inner membrane leaflet.[39,43,44]

CONCLUSION

Based on the information available for DesK and other HKs, we propose that DesK's internal mechanics trade off with mechanical inputs from the membrane. The protein tends to relax into a phosphatase state with a compact 2-HCC but

DOI: 10.1021/acs.accounts.6b00593
Acc. Chem. Res. XXXX, XXX, XXX−XXX

populates a less stable kinase state when forced by mechanical stress induced by the membrane. A similar idea has been proposed for the BvgS HK, whose phosphatase state would be the most stable form while the kinase state would be achieved by destabilization through activation of rotational dynamics.[37]

From the protein side, the initial cold signal at the TM domain must force rotation and separation of the 2-HCC helices. As discussed here, this is coupled to (i) a balance between helix hydration and helix−helix pairing in the TM domain (in the first half of TM1, second half of TM5 around Pro148 and the serine zipper, and in the 2-HCC), (ii) exposure/burial of hydrophobic residues from the DHp and 2-HCC (Figure 2), and, as we now introduce, (iii) possibly also to dynamics around conserved proline residues of the TM region. From the membrane side, the mechanical stress that acts on the TM domain upon temperature drop most likely combine changes in membrane thickness, fluidity, and water permeability.

As evidenced by the effects of several mutations in DesK, BvgS,[37] DctB,[45] and other HKs, the overall balance between protein and membrane mechanics is very delicate, such that small perturbations, either physiological signals or mutation-induced, lead to large shifts in the conformational equilibrium and thus clear-cut changes in catalytic output as required for fast adaptation to changing environments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.accounts.6b00593.

Analysis of structural distortions in DesKC structures, MD-based study of DesK TM helices in DOPC and DSPC membranes, and alignment of putative DesK HKs (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Matteo Dal Peraro. E-mail: matteo.dalperaro@epfl.ch.
*Diego de Mendoza. E-mail: demendoza@ibr-conicet.gov.ar.

ORCID

Diego de Mendoza: 0000-0002-9313-8118

Author Contributions

⊥L.A.A. and D.A. contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

M.D.P. is supported by the Swiss National Science Foundation (Grant Number 200020_157153). D.d.M. and D.A. are funded by the Agencia Nacional de Promoción Científica y Tecnológica (Awards PICT 2010-2613 and PICT 2014-2474), Argentina.

Notes

The authors declare no competing financial interest.

Biographies

Luciano A. Abriata is a biotechnologist and Ph.D. in Chemistry from the National University of Rosario, Argentina. He combines experiments and computation to understand biomolecular structure, dynamics, function, and evolution. He is a postdoctoral researcher at the Laboratory for Biomolecular Modeling at EPFL and SIB, Switzerland.

Daniela Albanesi received a Ph. D. in Biological Sciences in 2006 from the National University of Rosario, Argentina. She was a postdoctoral researcher at the Unit of Structural Biochemistry (nowadays, the Unit of Structural Microbiology) of the Pasteur Institute, Paris, France, and at present, she is a Career Investigator from CONICET and a teaching assistant at the University of Rosario, Argentina.

Matteo Dal Peraro received an M.Sc. in Physics at the University of Padova and a Ph.D. in Biophysics in 2004 at SISSA-ISAS, Trieste. He was a postdoctoral researcher at the University of Pennsylvania before joining EPFL, where he is Associate Professor at the Institute of Bioengineering, School of Life Sciences, leading the Laboratory for Biomolecular Modeling.

Diego de Mendoza is a Professor of Microbiology at the University of Rosario, Career Investigator from CONICET, and a group leader within the Instituto de Biologia Molecular y Celular de Rosario. de Mendoza holds a Ph.D. in Biochemistry from the University of Tucuman, Argentina, and he was a postdoctoral fellow at the University of Illinois at Urbana−Champaign, USA.

■ ACKNOWLEDGMENTS

L.A.A. thanks EMBO and the Marie Curie actions for an EMBO Long-Term Fellowship during which his research on DesK began. D.A. and D.d.M. are careers investigators of CONICET, Argentina. We thank Nathan Schmidt (UCSF) for help running accommodation index analyses.

■ ABBREVIATIONS

ABD, ATP-binding domain; DHp, dimerization and histidine phosphotransfer domain; 2-HCC, 2-helix coiled coil; MD, molecular dynamics; HK, histidine kinase

■ REFERENCES

(1) Capra, E. J.; Laub, M. T. Evolution of Two-Component Signal Transduction Systems. *Annu. Rev. Microbiol.* **2012**, *66*, 325−347.
(2) Nixon, B. T.; Ronson, C. W.; Ausubel, F. M. Two-Component Regulatory Systems Responsive to Environmental Stimuli Share Strongly Conserved Domains with the Nitrogen Assimilation Regulatory Genes ntrB and ntrC. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 7850−7854.
(3) Stock, A. M.; Robinson, V. L.; Goudreau, P. N. Two-Component Signal Transduction. *Annu. Rev. Biochem.* **2000**, *69*, 183−215.
(4) Russo, F. D.; Silhavy, T. J. The Essential Tension: Opposed Reactions in Bacterial Two-Component Regulatory Systems. *Trends Microbiol.* **1993**, *1*, 306−310.
(5) Stephenson, K.; Hoch, J. A. Two-Component and Phosphorelay Signal-Transduction Systems as Therapeutic Targets. *Curr. Opin. Pharmacol.* **2002**, *2*, 507−512.
(6) Gotoh, Y.; Eguchi, Y.; Watanabe, T.; Okamoto, S.; Doi, A.; Utsumi, R. Two-Component Signal Transduction as Potential Drug Targets in Pathogenic Bacteria. *Curr. Opin. Microbiol.* **2010**, *13*, 232−239.
(7) Bem, A. E.; Velikova, N.; Pellicer, M. T.; van Baarlen, P.; Marina, A.; Wells, J. M. Bacterial Histidine Kinases as Novel Antibacterial Drug Targets. *ACS Chem. Biol.* **2015**, *10*, 213−224.
(8) Ponting, C. P.; Schultz, J.; Copley, R. R.; Andrade, M. A.; Bork, P. Evolution of Domain Families. *Adv. Protein Chem.* **2000**, *54*, 185−244.
(9) Salazar, M. E.; Laub, M. T. Temporal and Evolutionary Dynamics of Two-Component Signaling Pathways. *Curr. Opin. Microbiol.* **2015**, *24*, 7−14.
(10) Mascher, T.; Helmann, J. D.; Unden, G. Stimulus Perception in Bacterial Signal-Transducing Histidine Kinases. *Microbiol. Mol. Biol. Rev.* **2006**, *70*, 910−938.

DOI: 10.1021/acs.accounts.6b00593
Acc. Chem. Res. XXXX, XXX, XXX−XXX

(11) Aravind, L.; Ponting, C. P. The Cytoplasmic Helical Linker Domain of Receptor Histidine Kinase and Methyl-Accepting Proteins Is Common to Many Prokaryotic Signalling Proteins. *FEMS Microbiol. Lett.* **1999**, *176*, 111−116.
(12) Gao, R.; Stock, A. M. Biological Insights from Structures of Two-Component Proteins. *Annu. Rev. Microbiol.* **2009**, *63*, 133−154.
(13) Anantharaman, V.; Balaji, S.; Aravind, L. The Signaling Helix: A Common Functional Theme in Diverse Signaling Proteins. *Biol. Direct* **2006**, *1*, 25.
(14) Zschiedrich, C. P.; Keidel, V.; Szurmant, H. Molecular Mechanisms of Two-Component Signal Transduction. *J. Mol. Biol.* **2016**, *428*, 3752−3775.
(15) Zhang, Y.-M.; Rock, C. O. Membrane Lipid Homeostasis in Bacteria. *Nat. Rev. Microbiol.* **2008**, *6*, 222−233.
(16) Saita, E. A.; de Mendoza, D. Thermosensing via Transmembrane Protein-Lipid Interactions. *Biochim. Biophys. Acta, Biomembr.* **2015**, *1848*, 1757−1764.
(17) Aguilar, P. S.; Hernandez-Arriaga, A. M.; Cybulski, L. E.; Erazo, A. C.; de Mendoza, D. Molecular Basis of Thermosensing: A Two-Component Signal Transduction Thermometer in Bacillus Subtilis. *EMBO J.* **2001**, *20*, 1681−1691.
(18) Aguilar, P. S.; Lopez, P.; de Mendoza, D. Transcriptional Control of the Low-Temperature-Inducible Des Gene, Encoding the delta5 Desaturase of Bacillus Subtilis. *J. Bacteriol.* **1999**, *181*, 7028−7033.
(19) Aguilar, P. S.; Cronan, J. E., Jr; de Mendoza, D. A Bacillus Subtilis Gene Induced by Cold Shock Encodes a Membrane Phospholipid Desaturase. *J. Bacteriol.* **1998**, *180*, 2194−2200.
(20) Cybulski, L. E.; Martín, M.; Mansilla, M. C.; Fernández, A.; de Mendoza, D. Membrane Thickness Cue for Cold Sensing in a Bacterium. *Curr. Biol.* **2010**, *20*, 1539−1544.
(21) Albanesi, D.; Martín, M.; Trajtenberg, F.; Mansilla, M. C.; Haouz, A.; Alzari, P. M.; de Mendoza, D.; Buschiazzo, A. Structural Plasticity and Catalysis Regulation of a Thermosensor Histidine Kinase. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 16185−16190.
(22) Schmidt, N. W.; Grigoryan, G.; DeGrado, W. F. The Accommodation Index Measures the Perturbation Associated with Insertions and Deletions in Coiled-Coils. Application to Understand Signaling in Histidine Kinases. *Protein Sci.* **2017**, *26*, 414−435.
(23) de Mendoza, D. Temperature Sensing by Membranes. *Annu. Rev. Microbiol.* **2014**, *68*, 101−116.
(24) Porrini, L.; Cybulski, L. E.; Altabe, S. G.; Mansilla, M. C.; de Mendoza, D. Cerulenin Inhibits Unsaturated Fatty Acids Synthesis in Bacillus Subtilis by Modifying the Input Signal of DesK Thermosensor. *MicrobiologyOpen* **2014**, *3*, 213−224.
(25) Martín, M.; de Mendoza, D. Regulation of Bacillus Subtilis DesK Thermosensor by Lipids. *Biochem. J.* **2013**, *451*, 269−275.
(26) Inda, M. E.; Oliveira, R. G.; de Mendoza, D.; Cybulski, L. E. The Single Transmembrane Segment of Minimal Sensor DesK Senses Temperature via a Membrane-Thickness Caliper. *J. Bacteriol.* **2016**, *198*, 2945−2954.
(27) Lee, A. G. Biological Membranes: The Importance of Molecular Detail. *Trends Biochem. Sci.* **2011**, *36*, 493−500.
(28) Kucerka, N.; Liu, Y.; Chu, N.; Petrache, H. I.; Tristram-Nagle, S.; Nagle, J. F. Structure of Fully Hydrated Fluid Phase DMPC and DLPC Lipid Bilayers Using X-Ray Scattering from Oriented Multilamellar Arrays and from Unilamellar Vesicles. *Biophys. J.* **2005**, *88*, 2626−2637.
(29) Blicher, A.; Wodzinska, K.; Fidorra, M.; Winterhalter, M.; Heimburg, T. The Temperature Dependence of Lipid Membrane Permeability, Its Quantized Nature, and the Influence of Anesthetics. *Biophys. J.* **2009**, *96*, 4581−4591.
(30) Inda, M. E.; Vandenbranden, M.; Fernández, A.; de Mendoza, D.; Ruysschaert, J.-M.; Cybulski, L. E. A Lipid-Mediated Conformational Switch Modulates the Thermosensing Activity of DesK. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 3579−3584.
(31) Bhate, M. P.; Molnar, K. S.; Goulian, M.; DeGrado, W. F. Signal Transduction in Histidine Kinases: Insights from New Structures. *Structure* **2015**, *23*, 981−994.
(32) Diensthuber, R. P.; Bommer, M.; Gleichmann, T.; Möglich, A. Full-Length Structure of a Sensor Histidine Kinase Pinpoints Coaxial Coiled Coils as Signal Transducers and Modulators. *Structure* **2013**, *21*, 1127−1136.
(33) Cybulski, L. E.; Ballering, J.; Moussatova, A.; Inda, M. E.; Vazquez, D. B.; Wassenaar, T. A.; de Mendoza, D.; Tieleman, D. P.; Killian, J. A. Activation of the Bacterial Thermosensor DesK Involves a Serine Zipper Dimerization Motif That Is Modulated by Bilayer Thickness. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 6353−6358.
(34) Zhang, S.-Q.; Kulp, D. W.; Schramm, C. A.; Mravic, M.; Samish, I.; DeGrado, W. F. The Membrane- and Soluble-Protein Helix-Helix Interactome: Similar Geometry via Different Interactions. *Structure* **2015**, *23*, 527−541.
(35) Saita, E.; Abriata, L. A.; Tsai, Y. T.; Trajtenberg, F.; Lemmin, T.; Buschiazzo, A.; Dal Peraro, M.; de Mendoza, D.; Albanesi, D. A Coiled Coil Switch Mediates Cold Sensing by the Thermosensory Protein DesK. *Mol. Microbiol.* **2015**, *98*, 258−271.
(36) Tao, W.; Malone, C. L.; Ault, A. D.; Deschenes, R. J.; Fassler, J. S. A Cytoplasmic Coiled-Coil Domain Is Required for Histidine Kinase Activity of the Yeast Osmosensor, SLN1. *Mol. Microbiol.* **2002**, *43*, 459−473.
(37) Lesne, E.; Krammer, E.-M.; Dupre, E.; Locht, C.; Lensink, M. F.; Antoine, R.; Jacob-Dubuisson, F. Balance between Coiled-Coil Stability and Dynamics Regulates Activity of BvgS Sensor Kinase in Bordetella. *mBio* **2016**, *7*, e02089.
(38) Audagnotto, M.; Lemmin, T.; Barducci, A.; Dal Peraro, M. Effect of the Synaptic Plasma Membrane on the Stability of the Amyloid Precursor Protein Homodimer. *J. Phys. Chem. Lett.* **2016**, *7*, 3572−3578.
(39) Lemmin, T.; Soto, C. S.; Clinthorne, G.; DeGrado, W. F.; Dal Peraro, M. Assembly of the Transmembrane Domain of E. Coli PhoQ Histidine Kinase: Implications for Signal Transduction from Molecular Simulations. *PLoS Comput. Biol.* **2013**, *9*, e1002878.
(40) Lemmin, T.; Dimitrov, M.; Fraering, P. C.; Dal Peraro, M. Perturbations of the Straight Transmembrane α-Helical Structure of the Amyloid Precursor Protein Affect Its Processing by γ-Secretase. *J. Biol. Chem.* **2014**, *289*, 6763−6774.
(41) Rauscher, S.; Pomès, R. Structural Disorder and Protein Elasticity. *Adv. Exp. Med. Biol.* **2012**, *725*, 159−183.
(42) Theillet, F.-X.; Kalmar, L.; Tompa, P.; Han, K.-H.; Selenko, P.; Dunker, A. K.; Daughdrill, G. W.; Uversky, V. N. The Alphabet of Intrinsic Disorder. *Intrinsically Disord. Proteins* **2013**, *1*, e24360.
(43) Goldberg, S. D.; Clinthorne, G. D.; Goulian, M.; DeGrado, W. F. Transmembrane Polar Interactions Are Required for Signaling in the Escherichia Coli Sensor Kinase PhoQ. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 8141−8146.
(44) Molnar, K. S.; Bonomi, M.; Pellarin, R.; Clinthorne, G. D.; Gonzalez, G.; Goldberg, S. D.; Goulian, M.; Sali, A.; DeGrado, W. F. Cys-Scanning Disulfide Crosslinking and Bayesian Modeling Probe the Transmembrane Signaling Mechanism of the Histidine Kinase, PhoQ. *Structure* **2014**, *22*, 1239−1251.
(45) Liu, J.; Yang, J.; Wen, J.; Yang, Y.; Wei, X.; Zhang, X.; Wang, Y.-P. Mutational Analysis of Dimeric Linkers in Peri- and Cytoplasmic Domains of Histidine Kinase DctB Reveals Their Functional Roles in Signal Transduction. *Open Biol.* **2014**, *4*, 140023.

DOI: 10.1021/acs.accounts.6b00593
Acc. Chem. Res. XXXX, XXX, XXX−XXX