have a more negative ϕ angle and endo pucker. Despite these differences at the isomeric proline, all of the structures are quite similar, with the largest global difference corresponding to rigid-body rotation of the CA^N protein by ~20° in a hinge motion about the ends of the cyclophilin-binding loop (**Fig. 1**). This apparent motion does not significantly alter CA^N–CypA contacts, nor does it strongly correlate with *cis* or *trans* conformations. Rather, the hinge angle is determined by different packing interactions for the independent complexes in the asymmetric unit: all A and A′ asymmetric unit complexes adopt one orientation, whereas all B and B′ complexes adopt the alternate orientation.

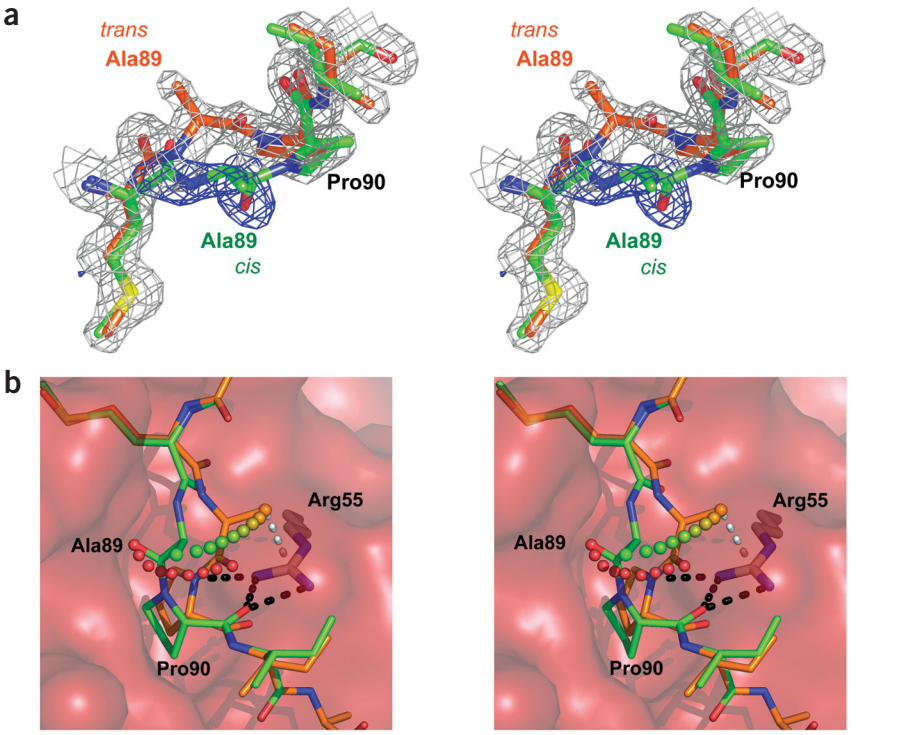

Figure 3 Proposed reaction pathway. (**a**) Mixed *trans* (80%) and *cis* (20%) structures of AMA-A. Maps were calculated before inclusion of the minor *cis* conformation in the model. $2F_o - F_c$ (silver) and $F_o - F_c$ (blue) maps are contoured at 1.0 and 2.0 × r.m.s. deviation, respectively. Final refined coordinates for the two partially occupied conformations are shown in orange (*trans*) and green (*cis*). (**b**) Top view of AMA-A *trans* (orange, 80% occupied) and *cis* (green, 20% occupied) conformations. Series of red and orange/green spheres show path of CA^N Ala89 O and Cβ atoms for intermediate conformations. This path would keep the Ala89 side chain clear of CypA protein and maintains a staggered conformation. White dashed line; contact between the side chain of CA^N Ala89 and CypA Arg55 that prevents CA^N Pro90 from binding fully into the active site when in the *trans* conformation. Black dashed lines represent the hydrogen bonds between CypA Arg55 and CA^N Pro90 O that prevent propagation of conformational changes to C-terminal residues and the hydrogen bond to CA^N Pro90 N that promotes catalysis.

Comparison of *cis* and *trans* conformations

An important finding from the crystal structures is that, relative to the central CA^N Pro90 residue, the C-terminal CA^N segment (Pro90–Pro93) adopts nearly the same conformation in all structures regardless of *cis* or *trans* conformation (**Fig. 2**). The largest difference between any of the 16 structures in a main chain ϕ or ψ angle over these residues is only 35°. The corresponding shifts in atomic positions are accommodated by variation in CypA–CA^N hydrogen bond lengths and solvent structure, including substitution of a direct hydrogen bond for a solvent-mediated interaction. The subsequent displacement of C-terminal residues seems to result from different positions of the loop ends caused by the variable CA hinge angle, and is allowed by the flat, open structure for this part of the active site cleft.

The situation is markedly different for residues N-terminal to CA^N Pro90. Although the overall path of the polypeptide is mostly unchanged, the switch between *cis* and *trans* conformations alters the main chain conformation for CA^N residues 88 and 89. The best overlap between *cis* and *trans* structures is observed for AAG-A (*trans*) and AMG-A (*cis*) (**Fig. 2c**). In going from *cis*- to *trans*-Pro90, CA^N residue 88 ψ angle and 89 ϕ and ψ angles change by 25°, 125° and 27°, respectively, whereas all other individual ϕ and ψ angles differ by no more than 10° for CA^N residues 75–105. Consequently, the structures overlap very closely both N- and C-terminal to the substrate proline, and the only substantial differences are confined to CA^N residues immediately N-terminal to the isomeric peptide. Conformational changes do not propagate to C-terminal CA^N residues because CypA binds Pro90 in a hydrophobic pocket and anchors the proline oxygen with two hydrogen bonds to the guanidinium of the essential CypA residue Arg55 (refs. 18,23,28) (**Fig. 2d**).

It is not obvious why the single substitution of A88M in CA^N results in a switch from *trans* to *cis* conformations for the AAG and AMG structures, because the CA^N residue 88 Cα atoms are within 0.08 Å of each other after least-squares overlap on CypA structures. The switch may result because the CA^N Met88 side chain adopts a slightly (~10°) altered orientation on the CypA surface, and presumably reflects the fine balance in energy observed for the bound *cis* and *trans* conformations[5,13].

Proposed reaction pathway

We favor a reaction pathway that requires minimal deviation from the ground-state crystal structures. Specifically, we propose that the proline remains essentially fixed relative to the enzyme, whereas the oxygen of the preceding residue rotates 180° in a clockwise direction (moving *cis* to *trans*) when viewed from the proline nitrogen atom (**Fig. 2d**). Catalysis will be enhanced by variables that disfavor the double-bond character of the peptide bond and favor the less polar and freely rotating single bond[29]. Thus, desolvation and the absence of compensating interactions for the peptide partial charges presumably enhance CypA catalysis by destabilizing the ground states. Additionally, formation of a hydrogen bond between Arg55 and the proline nitrogen would stabilize a pyramidal sp^3 hybridization state for the CA^N Pro90 nitrogen atom and resulting single-bond character for the peptide[23,30]. The distance between CypA Arg55 Nη1 and CA^N Pro90 main chain N atoms ranges from 3.3 to 4.4 Å in the ground-state complexes reported here, and is expected to shorten in the transition state to form a hydrogen bond between CypA Arg55 and the pyramidal sp^3-hybridized CA^N Pro90 N atom (**Fig. 2d**)[23].

A steric contribution to catalysis is also suggested by our structures of CA^N G89A complexes. Our decision to mutate CA^N Gly89 to alanine in the AAA construct was prompted by the observation that *trans* structures bind with CA^N Gly89 deep in the CypA active site cleft,