Structure, domain organization, and different conformational states of stem cell factor-induced intact KIT dimers

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Using electron microscopy and fitting of crystal structures, we present the 3D reconstruction of ligand-induced dimers of intact receptor tyrosine kinase, KIT. We observe that KIT protomers form close contacts throughout the entire structure of ligand-bound receptor dimers, and that the dimeric receptors adopt multiple, defined conformational states. Interestingly, the homotypic interactions in the membrane proximal Ig-like domain of the extracellular region differ from those observed in the crystal structure of the unconstrained extracellular regions. We observe two prevalent conformations in which the tyrosine kinase domains interact asymmetrically. The asymmetric arrangement of the cytoplasmic regions may represent snapshots of molecular interactions occurring during trans autophosphorylation. Moreover, the asymmetric arrangements may facilitate specific intermolecular interactions necessary for trans phosphorylation of different KIT autophosphorylation sites that are required for stimulation of kinase activity and recruitment of signaling proteins by activated KIT.

receptor tyrosine kinases | cell signaling | cancer | structure analysis | structural biology

A broad range of fundamental cellular processes, including proliferation, differentiation, survival, and metabolism, are mediated by signaling pathways that are activated by the 58 members of the receptor tyrosine kinase (RTK) family of membrane receptors (1). The receptor tyrosine kinase KIT was initially discovered as the viral oncogenic protein of a feline sarcoma virus (2). Subsequent studies have demonstrated that KIT and its ligand stem cell factor (SCF) play important roles in the control of proliferation, differentiation, and survival of a variety of cell types, including germ cells, hematopoietic cells, melanocytes, intestinal pacemaker cells, and sensory neurons in the CNS (3–5).

KIT and other members of type-III RTK family are composed of an extracellular ligand-binding region containing five Ig-like domains (designated D1–D5), followed by a single transmembrane (TM) spanning helix, a relatively large (35 aa) cytoplasmic juxtamembrane (JM) region, and a tyrosine kinase domain with a kinase insert region containing several autophosphorylation sites. Structural and biochemical studies have shown that the N-terminal region composed of D1, D2, and D3 of KIT extracellular region functions as a binding site for SCF, which, by virtue of its homodimeric structure, is responsible for bringing about KIT dimerization (6–11).

SCF-induced KIT dimerization results in homotypic contacts between the membrane proximal Ig-like domains D4 and D5 of two neighboring KIT molecules. These homotypic contacts position the TM and the cytoplasmic regions of KIT and other RTKs at a distance and orientation that facilitate tyrosine autophosphorylation, stimulation of enzymatic activity, and cell signaling (11–13). Oncogenic gain-of-function mutations in KIT have been identified in various cancers, including gastrointestinal stromal tumors, acute myeloid leukemia, melanoma, and mast cell malignancies (14–19). Most of the activating KIT mutations are located in the JM region, in D5 of the extracellular region, and in the tyrosine kinase domain.

In this study, we applied electron microscopy (EM) to obtain images of negatively stained preparations of SCF-stimulated KIT dimers. These images, together with previously described high-resolution X-ray crystal structures of SCF-induced dimers of KIT extracellular domain as well as KIT tyrosine kinase domains, provide valuable insight into ligand-induced dimerization and activation. Moreover, several forms of dimeric KIT molecules with different asymmetric arrangements of the two tyrosine kinase domains were identified. These asymmetric contacts may represent specific interactions occurring between two KIT tyrosine kinase domains poised toward trans autophosphorylation and activation.

Results and Discussion

Intact KIT was expressed in insect cells using the baculovirus expression system. The expression vector contains a signal sequence followed by a FLAG tag and the entire KIT sequence. We initially compared the level of expression and the homogeneity of different preparations of purified WT KIT, a KIT mutant with reduced tyrosine kinase activity (V603L), and a KIT mutant deficient in tyrosine kinase activity (K623A). Preliminary EM analyses revealed similar images of WT, V603L, and K623A KIT mutants. Because a significantly higher yield and a more

Significance

Stem cell factor (SCF) mediates its cellular responses by binding to and activating KIT, a transmembrane receptor tyrosine kinase. Here we describe an electron microscopy (EM) 3D reconstruction of negatively stained preparations of SCF-stimulated full-length KIT dimers. Assessment of the EM structure in respect to X-ray crystal structures of KIT extracellular and tyrosine kinase domains reveals the relative positioning of the individual domains in the context of the entire SCF-KIT complex. Whereas the homotypic contacts between the two KIT protomers show a consistent twofold symmetry for the ectodomains, the cytoplasmic arrangement is asymmetric and is found in several discrete conformations. The observed asymmetric contacts between tyrosine kinases may represent molecular interactions occurring during trans autophosphorylation and kinase stimulation.

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The authors declare no conflict of interest.

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homogenous preparation of the K623A KIT mutant were routinely obtained, most of the EM analysis described in this report was performed using this mutant. Moreover, no insect cell-derived tyrosine kinase-mediated phosphorylation of KIT mutants was detected.

Purified preparations of detergent-stabilized free KIT or KIT in complex with SCF (Fig. 1A) were placed on thin carbon films. These films were subsequently stained with a uranyl acetate or uranyl formate solution, respectively, followed by EM visualization of the negatively stained grids of free or SCF-occupied KIT particles.

**Two-Dimensional Analysis of Free KIT Monomers.** In contrast to ligand-activated KIT dimers (Fig. 1), analysis of the images of 2,021 particles of ligand-free KIT revealed an elongated “comet-like” structure (Fig. S1 A–C) of a size consistent with that of a KIT monomer. Based on the X-ray crystal structure of the intact free extracellular region of KIT (Fig. S1D) (11), the elongated thin region in particle images of monomeric KIT likely represents the extracellular region of the receptor. Similarly, the vast majority of particles showed an additional globular region that likely represents the cytoplasmic region containing the tyrosine kinase domain of KIT (Fig. S1 E and F).

Unfortunately, the class averages revealed a very high degree of flexibility in the orientation of the extracellular region relative to the intracellular region of monomeric KIT particles. This particle heterogeneity and the low contrast, which did not improve with the use of different stains (uranyl formate, molybdate, or tungstate), precluded a full 3D reconstruction of the ligand-free, monomeric KIT structure. Nevertheless, the consistently elongated appearance of the extracellular domain in the images of both free and ligand-bound KIT (Fig. 1 B and C) suggests that the ligand-binding portion of the extracellular region—composed of D1, D2, and D3 within the context of intact free KIT—is poised toward SCF binding and does not undergo large conformational changes on binding to KIT (11).

**Two-Dimensional Analysis of Dimeric SCF-KIT Complexes.** In contrast to monomeric KIT, addition of the ligand SCF resulted in much more homogeneous populations of particles that, based on their appearance, correspond to KIT dimers (Fig. 1B). Both the raw images and class averages showed a strong resemblance (Fig. 1 B and C) to the X-ray crystal structures of the intact extracellular region of KIT in complex with SCF (Fig. 1D) (11), which allowed an unequivocal assignment of both the ligand-bound extracellular region of KIT and the cytoplasmic regions of dimeric KIT molecules. Specifically, the EM images clearly revealed the overall domain organization and all salient features, including the relative orientations of D1 with respect to the rest of the ectodomain and the large cavity between D2 and D3 of adjacent monomers (Fig. 1D) (11).

To extend this analysis, we manually selected 12,612 particle tilt pairs (at 0° and 55°) using WEB (20). Reference-free alignment and classification of the tilt views into 100 classes with IMAGIC-5 (21) showed that most receptor dimers adopted a preferred orientation, lying sideways on the carbon support film, and exposing the distinctive twofold symmetry previously seen in the crystal structure of SCF-induced dimeric KIT extracellular complex (Fig. 1C) (11).

We next selected a representative class average and performed a reference-based alignment to bring all particles to the same in-plane orientation, followed by additional cycles of multireference alignment and classification to resolve particles into distinct classes representing unique subpopulations of KIT particles. This iterative procedure produced eight classes, most of which showed frontal views (Fig. 2A). In these views, the majority of KIT particles exhibited an approximate twofold symmetry along the entire long axis of the KIT particle (Fig. 2A, classes 1–6); however, a small population of KIT particles exhibited a significant bend involving an angle of ∼140° between the extracellular and cytoplasmic regions of KIT dimers (Fig. 2A, classes 7 and 8).

Whether the “bend” KIT configuration represents a genuine and biologically relevant conformational state or whether the bend is caused by sample preparation is unclear. Of note, however, this distinct conformation was sufficiently populated to allow calculation of a well-defined class average.

Along with this major conformational difference in the dimer population, we also observed differences in the shapes of the cytoplasmic regions (Fig. 2A, classes 1–5). Further analysis of these particle populations revealed significant variance in the particles in class 6 (Fig. 2A), and this class was not taken to the next step. Similarly, we decided to not pursue reconstruction of the “curved” receptor, because the biological significance of this configuration is currently unknown. The remaining classes (1–5) were sufficiently homogeneous and populated to allow 3D reconstruction by the random conical tilt method (20).

**Three-Dimensional Reconstruction of Dimeric SCF-KIT Complexes.** Separate 3D reconstructions of dimeric SCF stimulated KIT complexes were generated for each class from the datasets of the tilted particles. This resulted in reconstructions of two KIT forms (Materials and Methods) and revealed that particles belonging to class 3 could not be unambiguously assigned to either of the other two volumes. KIT form I (representing classes 4 and 5) and form II (representing classes 1 and 2) were reconstructed from 5,100 and 1,900 particles, respectively, out of a total of 12,612 particles in the dataset. The overall dimensions of forms I and II were similar, revealing an SCF-induced dimeric KIT complex

**Fig. 1.** EM images of SCF-induced KIT dimers. (A) Coomassie blue-stained SDS/PAGE analysis of purified unstimulated (Left) or SCF-stimulated KIT (Right). Protein bands corresponding to KIT and SCF are marked, and molecular weight markers are shown. The top four lanes are 250, 150, 100, and 75 kDa. (B) EM micrograph of individual SCF-induced KIT dimers attached to glow-discharged carbon film stained with 1% uranyl formate and imaged at 1.5 μm with a Philips Tecnai 12 electron microscope operating at 120 kV, using a 1 k × 1 k CCD camera. The EM micrograph shows the images of several single SCF-induced KIT particles (encircled) with a cavity similar to that seen in the crystal structure of the extracellular region of KIT in complex with SCF (D). (C) Thirty of 100 representative 2D class averages after reference-free alignment and classification. A total of 12,612 boxed particle images were subjected to 2D reference-free alignment, multivariate statistical analysis, and classification. Most classes show views consistent with preferential attachment of the SCF-induced KIT dimeric particles to the carbon film via the wide face of the extracellular dimeric KIT complex. (D) The typical architecture of crystal structure (11) of the extracellular region of KIT in complex with SCF is readily identified in the class averages. SCF is in magenta; D1, blue; D2, green; D3, yellow; D4, orange; D5, pink.
along the long axis of the structure, generated by the parallel seen in the KIT-SCF crystal structure, the extracellular regions of forms I and II of the intact reconstructed extracellular regions of KIT-SCF protomers on the extracellular side of the molecule. The total length of the SCF-induced KIT dimer and the length of extracellular or cytoplasmic portions of SCF-induced KIT dimers are marked. The extracellular, TM, and cytoplasmic KIT regions, as well as the membrane proximal D4 and D5, are also marked.

Extracellular Region of SCF-Induced KIT Dimers. The EM 3D reconstructions of the extracellular regions of both form I and form II exhibit a nearly perfect twofold symmetry, similar to that seen in the crystal structure of the dimeric KIT extracellular region (11). Moreover, the EM-derived structures of the two forms demonstrate that KIT dimerization is driven entirely by binding of the dimeric SCF molecule (6, 7), and that each SCF protomer forms a complex with a single KIT molecule by binding to the three N-terminal Ig-like domains D1, D2, and D3 (10, 11).

Structural analyses of SCF dimers have shown that complex formation between the two SCF protomers is mediated by a large interface involving primarily hydrophobic interactions (8, 9). Moreover, on KIT binding, the angle between the two SCF protomers is altered by \( \sim 5^\circ \) (10, 11). Inspection of the EM 3D reconstructions shows that the ligand-binding region of KIT composed of D1, D2, and D3 in complex with SCF appears in the reconstruction as a continuous density with a high contour level (Fig. 2C). This result is consistent with the existence of a large buried surface area of \( \sim 2,000 \, \text{Å}^2 \) for the interface formed between each SCF protomer and D1, D2, and D3 of KIT (10, 11). Both the crystallographic and EM analyses show that the interactions between SCF and KIT are mediated by a large interface and by close contacts. Moreover, the excellent fit between the crystal structures (10, 11) and the EM 3D reconstructions of SCF binding to KIT supports the EM data and allows selection of a reliable contour level for the entire KIT EM map (Fig. 3 and Fig. S2).

Homotypic D4 and D5 Contacts. Determination of the crystal structure of KIT extracellular region in complex with SCF and biochemical experiments with cells expressing WT or mutant KIT proteins have demonstrated that SCF-induced KIT dimerization leads to homotypic D4 and D5 contacts that are essential for receptor activation and stimulation of intracellular signaling pathways (11, 12). The homotypic D4–D4 contacts are positioned of the two KIT protomers that are bridged by SCF binding. Overall, the EM reconstructions of the extracellular regions of both form I and form II exhibit a nearly perfect twofold symmetry, similar to that seen in the crystal structure of the dimeric KIT extracellular region (11). Moreover, the EM-derived structures of the two forms demonstrate that KIT dimerization is driven entirely by binding of the dimeric SCF molecule (6, 7), and that each SCF protomer forms a complex with a single KIT molecule by binding to the three N-terminal Ig-like domains D1, D2, and D3 (10, 11).

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maintained by two salt bridges formed between Arg381, on the EF loop of D4 of one KIT protomer, and Glu386, located on a half-buried 3/10 helix upstream of the F strand on D4 of a second KIT protomer. The EM reconstruction of the D4–D4 contact region shows distinct densities consistent with the size and location of D4 within the KIT-SCF crystal structure (11). At high contour levels, the density of D4 becomes separated from the densities of D3 and D5, as expected given the small buried surface area of the D4–D3 and D4–D5 linker regions (Fig. 2C). Interestingly, the homotypic D4–D4 contacts mediated by the pair of salt bridges are clearly visible in the EM image as a strong continuous density bridging two D4 protomers as part of the density that separates the major and minor KIT cavities.

Although most of the EM 3D reconstructed structure of the extracellular region of KIT shows remarkable similarity to the crystal structure of the extracellular region of KIT, the homotypic D5–D5 contacts seen in the crystal structure do not fit into the volume of the EM 3D reconstruction (Fig. S3 A–C), and represent a genuinely unique structural feature not reported previously. Specifically, the D5 domains of each protomer required re-orientation and repositioning with respect to their crystallographically determined positions to fit the experimentally determined volume of the whole receptor molecule. Interestingly, rigid body repositioning of the D5 domains to conform with the receptor structure also gave rise to the observed minor cavity between D4 and D5 that otherwise could not be produced by simply docking the crystal structure into the volume. When contoured at higher level, the homotypic D5 contacts are depicted in the EM maps as a single continuous density, horizontal relative to the SCF plane and perpendicular to the D4 density. This implies that more robust homotypic D5 contacts are occurring in the context of intact dimeric KIT complex compared with the homotypic D5 contacts within the dimers of the isolated extracellular region (Fig. 3).

We surmise that the minor interactions occurring between D4 and D5 and in the linker connecting these two Ig-like domains (11) enable segmental flexibility within the D4–D5 hinge region. Moreover, the exceptionally long D strand may permit significant D5 mobility without interfering with DE loop (11) docking into its D4 groove, enabling freedom of movement of D5 relative to the rest of the extracellular KIT region. The best fit of D5 from the crystal structure into the EM map was achieved by rotating each D5 position by 60° and applying a tilt of 15° along the D4–D5 axis while maintaining strands A and G in the same interface as seen in the crystal structure (Fig. 3 A–C). This reorientation and repositioning brings D5 of two neighboring receptors closer together from a (main chain) distance of 15 Å in the KIT-SCF ectodomain crystal structure to a distance of 9 Å in the EM reconstructed KIT dimers. The close homotypic D5 contacts seen in the EM structure of intact KIT are likely caused by multiple homotypic interactions seen throughout dimeric KIT structures, including the TM and cytoplasmic regions (Fig. 2). Inspection of the density of this region using the contour applied to the EM reconstructed KIT dimers. The close homotypic D5 contacts seen in the EM structure of intact KIT are likely caused by multiple homotypic interactions seen throughout dimeric KIT structures, including the TM and cytoplasmic regions (Fig. 2). Inspection of the density of this region using the contour applied to the EM reconstructed KIT dimers. The close homotypic D5 contacts seen in the EM structure of intact KIT are likely caused by multiple homotypic interactions seen throughout dimeric KIT structures, including the TM and cytoplasmic regions (Fig. 2). Inspection of the density of this region using the contour applied to the EM reconstructed KIT dimers.
kinase dimers have a dimension of 80–90 Å along the membrane vertical axis, a distance consistent with the size of the cytoplasmic EM density. Although these docking analyses do not provide information about molecular contacts among the asymmetric kinase dimers that have been successfully docked into the EM volumes, common features of all docked asymmetric dimers are that the N lobe of the membrane proximal kinase domain is located in the vicinity of the TM region close to the cell membrane, and the C lobe of the membrane proximal kinase domain is responsible for mediating asymmetric contacts with the membrane distal tyrosine kinase domain.

Although the biological and mechanistic relevance of the observed asymmetry awaits further exploration, our reconstruction suggests that the asymmetry reflects structural intermediates in which the two tyrosine kinase domains are captured before the initiation of trans phosphorylation. Moreover, the possibility that distinct asymmetric arrangements can be trapped may provide a mechanism for the well-defined order of the trans autophosphorylation reaction (28, 29).

Intriguingly, the tyrosine autophosphorylation sites of KIT and other RTKs are located on different parts of the cytoplasmic region in the catalytic tyrosine kinase domain, the kinase insert region, the JM domain, and the C-terminal tail of the receptor. This suggests that the two kinases interact alternately through several different asymmetric arrangements, contacts that function as successive checkpoints along the trajectory of autophosphorylation, kinase activation, and cell signaling. In other words, to phosphorylate tyrosines separated from one another by distances as large as 50 Å in the cytoplasmic region (28), one kinase molecule will first function as an active enzyme, and a second kinase molecule will first function as a substrate (Fig. 4), after which the two kinase molecules will switch roles. Accordingly, the kinase that functions as an active enzyme will sample the surface of the inactive kinase to identify specific docking sites on the substrate kinase. Sequential docking from one docking site to the next site will facilitate efficient trans phosphorylation of all tyrosine residues in the inactive substrate kinase in an orderly manner.

It follows that during autophosphorylation, several distinct asymmetric transient arrangements between an activated kinase and the substrate kinase must be formed. A potential asymmetric arrangement may be mediated by interactions between a membrane proximal kinase that functions as a substrate with a membrane distal kinase that exists in an active configuration and functions as an enzyme (28). Structural and biochemical studies have shown that the JM region interacts with the tyrosine kinase domain of KIT (and other RTKs), including the activation loop, to stabilize an autoinhibited “closed” kinase configuration. Furthermore, cis autoinhibition is stably relieved by phosphorylation of a tyrosine or by oncogenic mutation in the JM region, resulting in an activated “open” configuration of the kinase domain.

Finally, Fig. 5 shows a model for the overall structure of SCF-induced KIT dimers, in which the EM 3D volume of the dimeric extracellular region and the EM 3D volume of form II of the cytoplasmic KIT dimeric structure were combined with high-resolution corresponding KIT crystal structures to highlight the symmetric architecture of the ligand-bound extracellular regions and the asymmetric architecture of the cytoplasmic regions.

**Materials and Methods**

**KIT Expression Vector.** The region encoding for amino acids 33–972 of human KIT (lacking the signal sequence and an additional seven amino acids) was amplified. The stop codon was replaced by a sequencing for Gly-Ser followed by six histidines and a stop codon using the forward primer GTACCATGGGGAAACGCTCCACCA and the reverse primer GCTCTAGATCGTGATGTTGGTGATGGTGATGCGATCCGACATCGTCGTGCACAAGCA. The PCR product was digested with NcoI and XbaI and ligated into a modified pFastBac plasmid, designated pK309-9 (a gift from Kari Keinanen, University of Helsinki).

**Fig. 4.** Asymmetric dimers from the crystal structure of the tyrosine kinase domains of KIT or FLT3 fitted into the EM 3D volume of KIT-SCF form II complex. Dimers derived from the lattice of the crystal structure of the tyrosine kinase domain of KIT (PDB ID code 1T45) (A) or FLT3 (PDB ID code 1RJB) (B) display a similar asymmetric arrangement and fit into the EM 3D volume of KIT-SCF complex form II (gray). In both panels, the membrane proximal kinase domain is in green and the membrane distal kinase is in blue. The JM region of the membrane proximal kinase is folded over the nucleotide-binding site, and amino acids from the JM region of the membrane distal kinase form contacts with the membrane proximal kinase.

**Fig. 5.** A model for the structure of SCF-induced dimeric KIT complex combining the EM 3D volume of form II with high-resolution KIT crystal structures. The crystal structure of SCF-induced extracellular KIT dimer (PDB ID code 2E9W) is fitted into the EM 3D volume. SCF is in magenta; D1, blue; D2, green; D3, yellow; D4, orange; D5, pink. A large adjustment in the D5 contacts is required to fit this region into the EM 3D volume of the SCF-induced KIT volume. A symmetry-related structure of the tyrosine kinase of KIT crystal lattice (PDB ID code 1T45) is fitted into the EM 3D volume of the cytoplasmic region of SCF-induced KIT form II complex. The fitted membrane proximal tyrosine kinase domain is in green (light green for N lobe and dark green for C lobe), and the membrane distal tyrosine kinase domain is in blue (light blue for N lobe and dark blue for C lobe). (B) Top view (perspective indicated in A by the eye symbol) over the crystal structure of the extracellular KIT-SCF complex fitted into the EM 3D volume of SCF-induced KIT form II complex. (C) Bottom view (perspective indicated in A by the eye* symbol) over the crystal structure of the KIT kinase domain fitted into the EM 3D volume of SCF-induced KIT form II complex.
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Sample Preparation and EM. Protein samples were diluted, and a 5-μL drop was placed on a glow-discharged carbon film-coated holey carbon copper grid. Protein was absorbed for 1 min and then stained repeatedly, each time for 1 min, by floating the grid onto 100-μL 1% uranyl formate drops. The grid was then gently blotted, leaving a residual amount of uranyl formate to dry. EM images were recorded using a 1k × 1k Gatan CCD camera on a Tecnai 12 transmission electron microscope equipped with a LaB6 filament and operated at 120 kV. Images were collected at an ~1.3-μm mean underfocus and a nominal magnification of ~30,000, which yielded an effective pixel size of 5.5 Å at the level of the specimen.

For the KIT-SCF complex, 12,612 particles were interactively selected using WEB from 393 tilt pair micrographs, collected at 0° and 55°. For KIT monomer, 2,021 particles were selected from 400 micrographs using SIGNATURE (30). The chosen particles were normalized and low-pass filtered, followed by multivariate statistical analysis, classification, and alignment (20). After three rounds of multireference alignment, final classification resulted in 50 unique classes of monomeric KIT particles (Fig. S1 C). Particles were extracted to a particle window size of 144 × 144 pixels. One cycle of reference-free 2D alignment and classification was first performed on the untilted set of KIT-SCF particles using IMAGIC (21). The KIT monomer dataset was further subjected to iterative cycles of multivariate statistical analysis and multireference alignment with IMAGIC. The KIT-SCF complex set was further subjected to three rounds of multireference alignment and K-means classification using SPIDER, resulting in a total of eight unique classes. A 3D volume was calculated for each of the eight classes by back-projecting the corresponding particles from the tilted set. The resulting volumes were subjected to translational, rotational, and angular refinement and were visually compared with one another using UCSF Chimera, which revealed that class pairs 1-2, 4-5, and 7-8 described identical volumes. The matched classes (1-2 and 4-5) were pooled and subjected to 3D reconstruction by projection matching, with starting models that were low-pass filtered to 50 Å.

Next, the particles of classes 1-5 were pooled and subjected to iterative projection, matching rounds against the two volumes competitively. Images were assigned to the reference volume that yielded the higher correlation coefficient. In a final step, the set of untilted particles was added to the tilted set for 3D reconstruction. The resulting form I (derived mainly from classes 4 and 5) and form II (derived mainly from classes 1 and 2) were reconstructed from 5,100, and 1,900 particle pairs, respectively. The final resolution of the final 3D maps was 25 Å for form I and 28 Å for form II, according to a Fourier shell correlation curve of 0.5 (Fig. S2).

Docking of Crystal Structures and Molecular Graphics. Docking of crystal structures into EM densities was performed using COOT and UCSF Chimera. Images were produced using PyMol and UCSF Chimera.

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Supporting Information

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SI Materials and Methods

**KIT Expression Vector.** The region encoding for amino acids 33–972 of human receptor tyrosine kinase (KIT) (lacking the signal sequence and additional seven amino acids) was amplified. The stop codon was replaced by a sequence encoding for Gly-Ser, followed by six histidines and a stop codon using the forward primer GTACCATGGGGGAACCGTCTCCACCA and the reverse primer GCTCTAGATCAGTGATGGTGATGGTGATGCGA TCCGACATCGTCGTGCACAAGCA. The PCR product was digested with NcoI and XbaI and ligated into a modified pFastBac plasmid, designated pK503-9 (a gift from Kari Keinanen, University of Helsinki, Helsinki). The plasmid contains a signal sequence from ecdysteroid UDP-glucosyltransferase (EGT), followed by a FLAG tag and a cloning site. Site-directed mutagenesis was used to generate expression vector for a KIT mutant with reduced tyrosine kinase activity (V603L) and a for a KIT mutant deficient in tyrosine kinase activity (K623A). The baculovirus system was used according to procedures described in the Bac-to-Bac instruction manual (Invitrogen), and the third viral passage (P3) was used for KIT expression.

**KIT Expression and Purification**

Insect Sf9 cells were grown in ten 2-L roller bottles, each containing 50 mL of Grace’s insect medium supplemented with 10% heat-inactivated FBS at 27 °C. After 6 d, on reaching complete cell confluence, the medium was replaced, and the cells were infected with recombinant baculovirus. After 42 h, the cells were harvested; suspended in 50 mL lysis buffer solution containing 200 mM NaCl, 20 mM Hepes pH 7.4, 0.5 mM TCEP, 1 mM EDTA, a protease inhibitor mixture (Roche), and 10% glycerol; and then lysed using a French press. Cellular debris and organelles were spun down by low-velocity (600 × g) centrifugation for 10 min, after which the supernatant was subjected to ultracentrifugation (Beckman 45Ti) for 45 min at 42,000 rpm.

Using a Dounce homogenizer, the crude membrane pellet was homogenized in a buffer solution containing 200 mM NaCl, 20 mM Hepes pH 7.4, 5% glycerol, a protease inhibitor mixture (Roche), and 250 mM sucrose and then flash-frozen by spraying the suspension into liquid nitrogen. The frozen membranes were maintained at −80 °C at a protein concentration of 4 mg/mL until use. Membrane solubilization was carried out at protein concentrations of 2 mg/mL for 4 h at room temperature in solubilization buffer containing 200 mM NaCl, 20 mM Hepes pH 7.4, 5% glycerol, a protease inhibitor mixture (Roche), and 1% N-dodecyl-β-D-maltopyranoside (DDM). KIT-SCF (stem cell factor) complexes were prepared by adding to suspended membrane preparations 200 μg of SCF for 12 h at 4 °C before the addition of DDM.

Solubilized membrane preparations were cleared by ultracentrifugation with a Beckman 45Ti at 42,000 rpm for 45 min, followed by the addition of a CaCl₂ solution to a final concentration of 3 mM together with 500 μL of pre-equilibrated M1 anti-FLAG mAb beads (Sigma-Aldrich) for 2 h at 4 °C. The beads were packed into a Poly-Prep column (Bio-Rad) and washed with a buffer solution containing 200 mM NaCl, 20 mM Hepes pH 7.4, 5% glycerol, 0.05% DDM, and 3 mM CaCl₂. KIT preparations and KIT-SCF complex preparations were eluted into 10 200-μL fractions of with elution buffer containing 200 mM NaCl, 20 mM Hepes pH 7.4, 5% glycerol, 0.05% DDM, and 3 mM EDTA, followed by SDS/PAGE analysis. Protein aggregates that were detected in preliminary electron microscopy (EM) analysis were almost completely eliminated by ultracentrifugation for 16 h at 4 °C in a 10–25% glycerol gradient with a 0–0.1% glutaraldehyde gradient using a SW41Ti rotor (1). The glycerol gradient tubes were fractionated into 0.5-mL fractions and analyzed by SDS/PAGE.

Fig. S1. EM images of monomeric KIT. (A) Electron micrograph of negatively stained full-length KIT monomers stained with uranyl formate. (Scale bar: 100 nm.) (B) Gallery of individual monomeric KIT particles, windowed and aligned from several micrographs. Monomeric KIT particles are composed of an elongated stem-like structure connected to a globular structure. (C) Twenty-five representative class averages (out of 50) of monomeric KIT particles exhibiting a high degree of structural heterogeneity. (D) The elongated comet-like structure of KIT particles is similar to the high resolution crystal structure of the extracellular region (in surface representation) of KIT (PDB ID code 2EC8) composed of five Ig-like domains: D1 (blue), D2 (green), D3 (yellow), D4 (orange), and D5 (pink). (E) Surface representation of the crystal structure of the tyrosine kinase domain of KIT (PDB ID code 1T45). The N lobe of the kinase is in light green, and the C lobe is in dark green. The juxtamembrane (JM) domain is shown as a red tube folded in part into the cleft formed between the two kinase lobes. (F) Schematic representation of KIT. The extracellular, transmembrane (TM), and tyrosine kinase regions, including the JM domain, are shown in cyan, yellow, and green, respectively. High-resolution crystal structures of the extracellular region (before and after SCF stimulation) and the tyrosine kinase domain (before and after activation) were described. The structures of the TM, kinase insert region, and C-terminal tail (in gray) were not described.
**Fig. S2.** Euler angle distribution (Right) and Fourier shell correlation (FSC) plots of form I (Upper) and form II (Lower) reconstructions. The 0.5 threshold of FSC curves corresponds to 25.5 Å and 28.8 Å, respectively.

**Fig. S3.** Model for the structure of SCF-induced dimeric KIT complex combining the EM 3D volume of form I with high-resolution KIT crystal structures. The crystal structure of SCF-induced extracellular KIT dimer (PDB ID code 2E9W) was fitted into the EM 3D volume and color-coded as in Fig. 5. A symmetry-related structure of the tyrosine kinase of FGFR2 crystal lattice (PDB ID code 2PVF) was fitted into the EM 3D volume of the cytoplasmic region of SCF-induced KIT form I complex and color-coded as in Fig. 5 B and C.