Subcellular localizations and molecular functions of Rab11 proteins

Rab11a localizes to the endocytic recycling compartment (ERC)/recycling endosome [1][2][3] and trans-Golgi network (TGN) compartment [4] to control the vesicle trafficking through and from these compartments [5]. Rab11a controls tissue homeostasis during both embryonic development and postnatal periods [6][5]. Recently, Yan *et al.* showed that Rab11a regulates vascular endothelial-cadherin protein recycling to endothelial cell plasma membrane [7]. Intestinal Rab11a is also required for the apical protein localization [6].

Rab11b also localizes to ERC [5] and regulates the recycling of transferrin receptor to the plasma membrane [8]. Rab11b regulates exocytosis in neurons and neuroendocrine cells [9]. However, little is known about the functional differences between Rab11a and Rab11b. They localize to different vesicle compartments in gastric parietal cells [10]. Silvis *et al*. have shown that Rab11b, but not Rab11a, specifically regulates the recycling of the intracellular cystic fibrosis transmembrane conductance regulator (CFTR) in polarized epithelial cells in the intestines [11][5]. Later, Haugsten *et al.* have shown that Rab11a and Rab11b may play slightly different roles in fibroblast growth factor receptor 4 (FGFR4) recycling [12]. While knockdown of Rab11a, Rab11b or Rab11a/b simultaneously reduced FGFR4 transport out of the ERC, knockdown of Rab11b alone but not Rab11a, accumulated FGFR4 in a perinuclear compartment.

Ras and Rab1 proteins

Previously, we have identified novel binding sites in Ras and Rab1 proteins [13][14]. Ras is a family of proteins in the Ras superfamily of proteins that regulates signaling pathways that control gene expression of cell growth, differentiation and survival. Three members of this family: K-Ras, H-Ras and N-Ras, are frequently mutated in cancer and hyper-proliferative

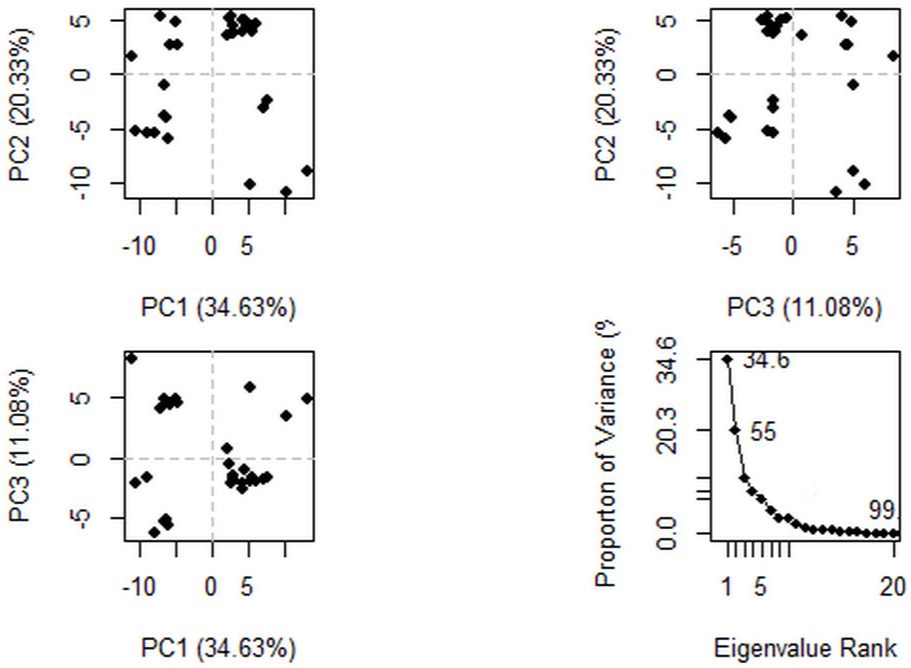
developmental disorders [15]. K-Ras localizes to cytosol and plasma membrane. H-Ras and N-Ras localizes to golgi apparatus and plasma membrane. These isoforms share more than 85% identity. Through computational and experimental methods, we have previously identified three allosteric pockets and inhibitors for Ras [14]. Rab1 is a member of the Rab GTPase family that regulates membrane trafficking pathways that are related to transport between endoplasmic reticulum and golgi apparatus, and autophagy [16]. Rab1 localizes to ER, GA and early endosome [16][17]. It has two isoforms, Rab1a and Rab1b, that share 92% of sequence identity [18]. Rab1 is associated with various human cancers including prostate cancer [19], triple-negative breast cancer (TNBC) [20], colorectal cancer [21] and tongue cancer [22]. Aberrant expression of Rab1 is also associated with diseases such as cardiac hypertrophy [23] and Parkinson's disease [24].

Principal Component Analysis (PCA), Independent Component Analysis (ICA) and Locally Linear Embedding (LLE)

PCA and ICA are linear dimensionality reduction techniques. PCA projects data from high dimensional space to low dimensional space such that the variance of data is maximized, assuming that the direction with the biggest variance is the most important [25][26]. ICA performs dimensionality reduction by deriving independent components from the high dimensional data in such a way that maximizes non-Gaussianity [26]. While PCA minimizes co-variance of data, ICA minimizes mutual information of data [25]. LLE is a manifold learning algorithm [27] which is used for non-linear dimensionality reduction. LLE can identify the underlying structure of the manifold better than PCA and ICA [28].

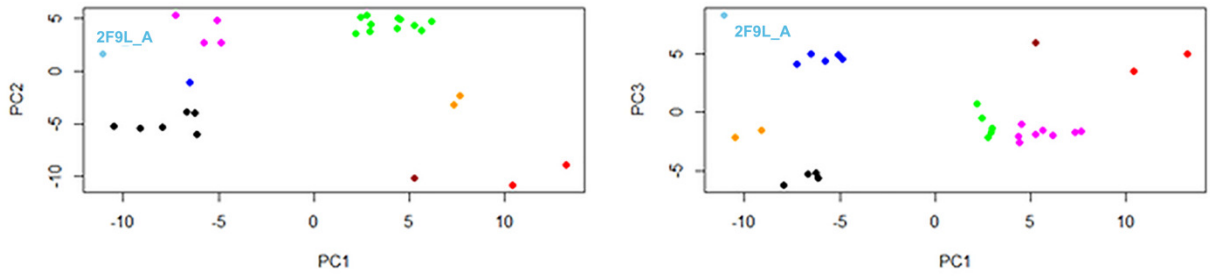
PCA and Dynamical Cross Correlation Analysis (DCCM) analysis on the ensemble of 28 Rab11 structures

We first performed PCA on the ensemble of 28 structures. More than 80% of the variance is captured in the first three principal components (PCs) (S1.1 Fig).



S1.1 Fig. Results of PCA on the ensemble of 28 Rab11 structures.

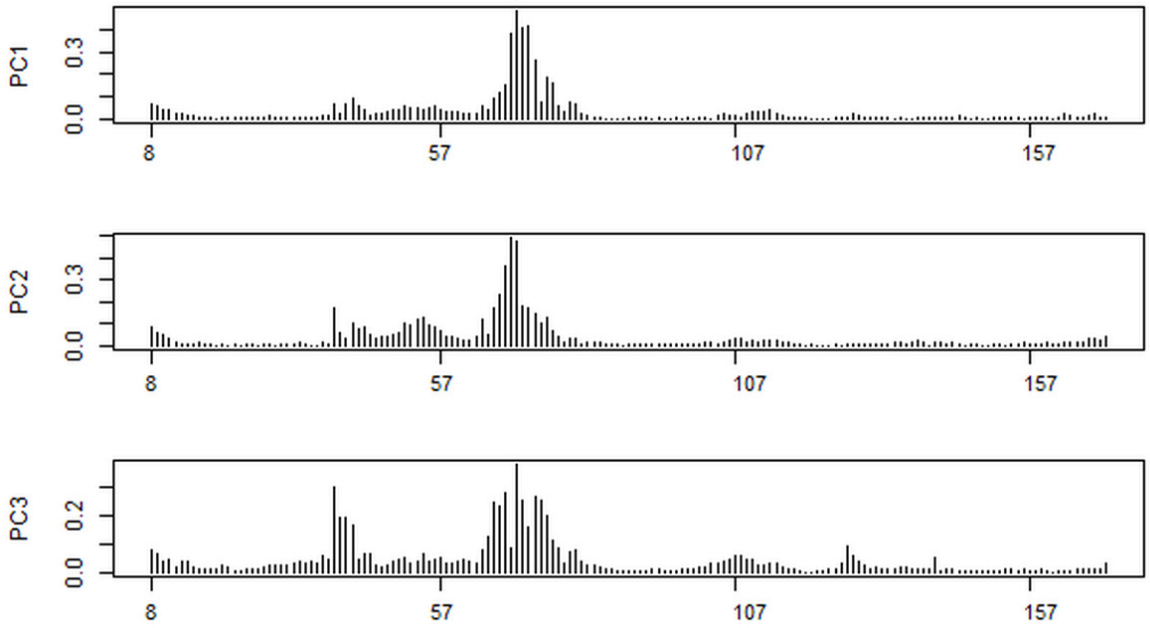
We projected the structures in the Rab11 ensemble onto the first two PCs and, the first and third PCs. More than 60% of the variance is captured in these PCs (S1.2 Fig). We observed that 2F9L_A is separated from other structures.



S1.2 Fig. Projection of structures from the ensemble of 28 structures onto the PC space.

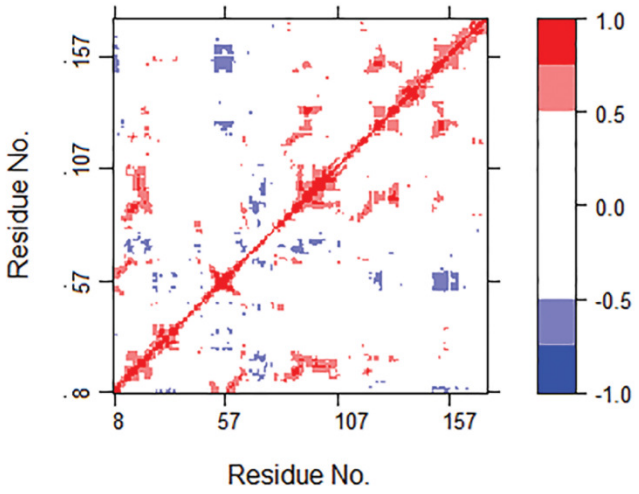
PDB entry 2F9L_A (labeled) that lacks residues E39-K41 is separated from all other structures.

On examining the contribution of each residue to the first three principal components, we observed that the largest contributions are made by residues in switch 1 (E39-V46), switch 2 (A68-A79) and interswitch (E47-T67) regions of Rab11 (S1.3 Fig).



S1.3 Fig. Residual contributions of Rab11 structures in PC1, PC2 and PC3.

Furthermore, Dynamical Cross-Correlation Matrix (DCCM) analysis [29] of the superposed Cartesian coordinates of C_α atoms of Rab11 structures shown that there are correlated motions within these regions (S1.4 Fig). Since switch 1 region is found to be important in Rab11, we excluded 2F9L_A which lacks coordinates of this region from further analyses.



S1.4 Fig. Cross-correlated motions in Rab11. Red and blue colors represent positive and negative correlations, respectively. Motion occurring along the same direction is represented by positive correlation (red), whereas motion occurring along opposite directions is represented by negative (anti-) correlation (blue). Positive correlations with values greater than 0.5 and negative correlations with values less than -0.5 are shown.

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