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doi:10.1017/S1431927619007293
Microsc. Microanal. 25 (Suppl 2), 2019

Structural Studies αvβ8 Integrin by Single Particle Cryo-EM

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Integrins comprise a diverse family of protein receptors involved in bi-directional signaling across the plasma membrane. They are responsible for numerous cellular processes associated with homeostasis, immunity, tissue repair and neoplasia [1]. Integrins are heterodimers, assembled from an α and a β subunit, which are non-covalently interacting at the α- and β-head domain. Each subunit contains a flexible leg, a single transmembrane helix and a short cytoplasmic domain. In vertebrates, eighteen α- and eight β-subunits assemble into 24 different integrin heterodimers with various tissue distribution, binding properties, and biological functions. The prevailing model of integrin activation involves large-scale conformational changes, with three discrete conformations, reminiscent of the opening of a 'switch-blade'. According to this model, integrins are proposed to exist in a compact, inactive and bent conformation, which undergoes complete reorganization to an extended conformation upon activation. The extended conformation is further subdivided into extended closed and extended open states that have low and high binding affinity for their physiological ligands, respectively. The 'switch-blade' model, which was derived from structural analysis of β2 and β3 integrins, may not as generalizable as previously suggested, because recent studies suggested different integrin classes undergo distinct conformational changes. For instance, β1 and β4 integrins have been proposed to adopt five conformational states with sub-classification of the bent conformation into acutely bent, half bent, and slightly bent, whereas αvβ8 exhibit a single, extended conformation [2,3].

We recently determined multiple sub-nanometer single particle cryo-electron microscopy (cryo-EM) structures of αvβ8 integrin corresponding to conformational snapshots of this extended integrin as it surveys the environment for its ligand, Latent Transforming Growth Factor–β (LTGF-β) [4]. Understanding αvβ8 integrin is of exceptional interest as a way of selectively targeting TGF-β activation. Preclinical studies have implicated αvβ8 as a promising drug target for both fibroinflammatory disease and tumor growth progression in cancer [3,5]. The current lack of high-resolution structural information for αvβ8 integrin limits our understanding of the molecular basis of its reduced conformational landscape, compared to other integrins, and of the resulting functional implications. However, improving the resolution of αvβ8 integrin structure is very challenging, as its flexible leg domains causes considerable conformational heterogeneity, hindering achieving high-resolution structure of the entire ectodomain. Our strategy of improving the resolution of integrin structure is to apply extensive classification and focused alignment to regions that are more stable for high-resolution structure determination. Here, we describe our progress towards improving the

resolution of our single particle cryo-EM studies of αvβ8 integrin, aiming to obtain a detailed understanding of αvβ8 integrin signaling.

We used single particle cryo-EM to study a complex of the ectodomain of integrin αvβ8 bound with two conformational specific fragments of antigen binding (Fab) that do not disrupt activation or ligand binding. This strategy of binding Fabs with conformational epitopes to target proteins has proven to be an effective way to aid in particle alignment of small and flexible proteins by increasing the molecular weight of the imaged sample and serving as a fiducial for computational alignment of particle images during data processing [6]. In the case of αvβ8/68-6B8 complex, even with two Fabs bound to the integrin ectodomain, there is still a considerable amount of conformational heterogeneity in the leg regions. Through extensive classification and focused refinement, we improved the resolution of the αvβ8 integrin headpiece to 3.9Å, which allows for accurate modeling. Detailed structural modeling will provide important details towards the understanding of integrin binding and activation.

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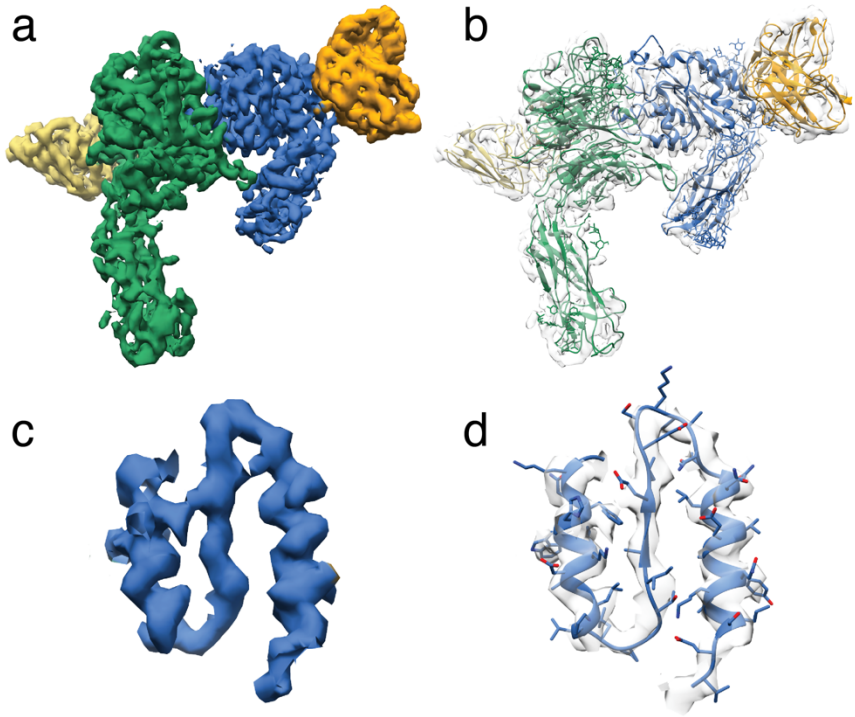

Figure 1. a, Cryo-EM structure of αvβ8-68-8B8 complex at a resolution of 3.9 Å resolution. The alpha subunit is shown in green, the beta subunit is shown in blue, Fab 68 is shown in orange and Fab 8B8 is shown in gold. b, The atomic model of the headpiece shown in ribbon format, fitted into the cryo-EM density. The headpiece portion shown consists of the αv head and thigh, and the β8 βI and hybrid domains. c, d, Close-up of the β8 βI domain showing clear helical pitch and bulky side chains.

https://doi.org/10.1017/S1431927619007293 Published online by Cambridge University Press