Nanobody-Displaying Flagellar Nanotubes

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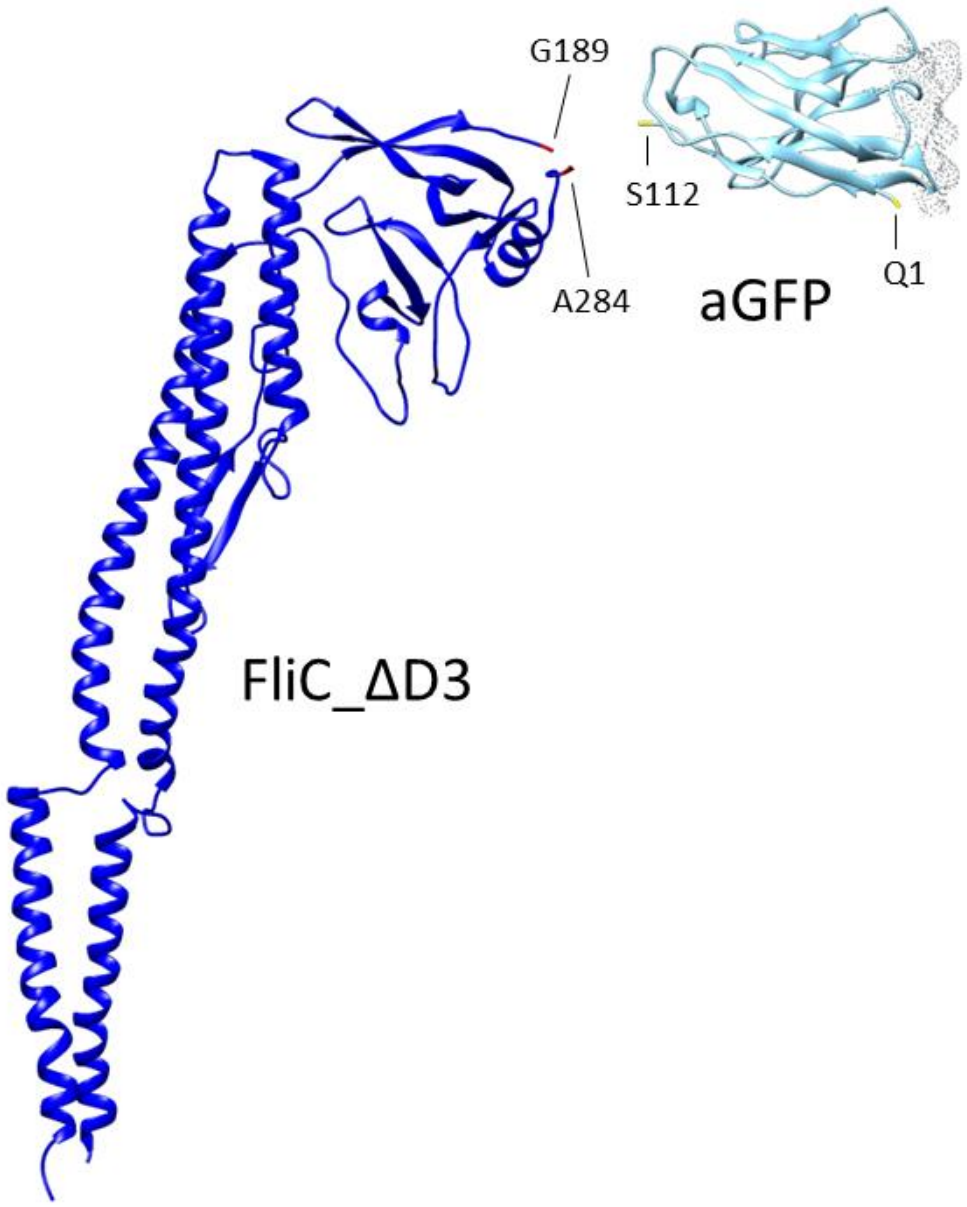
	Linkers
GROUP 1	VQQKYKVSDTAATVTG LEPRVFREVTVKGI VIGYMPIPVGVA FSSQKGSVGYA AKIYAELVGFG LSGEYDKAV VSKGYEKA TGEQGLG LPEEKG ALEG GSGM NVG
GROUP 2	**GVTPSTTALPDIVNLSTNYLDKNTREDRIHSIKDF** GVTPSTTALPDIVNLSTNYLDKNTREDRIH GVTPSTTALPDIVNLSTNYLDKNTRE GVTPSTTALPDIVNLSTNYLDKN GVTPSTTALPDIVNLSTNYL GVTPSTTALPDIVNLSTN GVTPSTTALPDIVNLS GVTPSTTALPDIV GVTPSTTA GVTPST GVTPS GVT GV
GROUP 3	**SAAAATPAVRTVPQYKYAAGVRNPQQHLNAQPQVTMQQPAVHVQGQEPL** SAAAATPAVRTVPQYKYAAGVRNPQQHLNAQPQVTMQQPAVHV SAAAATPAVRTVPQYKYAAGVRNPQQHLNAQPQVTMQQPA SAAAATPAVRTVPQYKYAAGVRNPQQHLNAQPQV SAAAATPAVRTVPQYKYAAGVRNPQQHLNA SAAAATPAVRTVPQYKYAAGVRNPQQ SAAAATPAVRTVPQYKYAAGVRN SAAAATPAVRTVPQYKYAAG SAAAATPAVRTVPQYKYA SAAAATPAVRTVPQY SAAAATPAVRTV SAAAATPAV SAAAAT SAAA SAA SA
GROUP 4	GTTTTATTMGAMTQNENTAGMGNAQQHLHAQTQMAVQQTAMHV CTATAATAVGAVAQDKNTAGVRHTQQHLNTQAQM GMATRAATLTDIMNLGAHNLDKNAREDGIH CTATAATAVCAMTQDEDTARVRHAQQ RMTSGAATLAYIMHLGANNLNEH CTATAATAMSAMAQNKDTAG SVTASAATLANVMHLRAN STATAATAVRAMTQD LKAGVFGEVAVKGV RMAARAATLTNIV TEIDAKLVGFR CTATAATAM LGREDDETV AREQGLR GVATRA RRRM RTAT DMR RVA RA

Supplementary Table S1.

Linker segments used for the insertion of the aGFP_ENH single-domain antibody into the variable middle part of flagellin. Group1 contains linkers of various lengths and conformational properties selected from the linkerdbwww linker database (http://www.ibi.vu.nl/programs/linkerdbwww/). Linkers in Group2 and Group3 are derived by successive truncation of two long linkers (shown in bold) obtained from the linkerdbwww database. Random oligopeptides (Group4) were also applied as potential linker segments.



Supplementary Figure S1.

The polypeptide backbone of flagellin (blue) after removal of the D3 domain (FliC_ΔD3). The resulting free ends of the D2 domain (G189 and A284) are close to each other, separated by about 6 Å. The N- and C-terminal ends of the aGFP single domain antibody (light blue) are at the opposite sides of the molecule. Suitable linker segments are required for functional insertion of aGFP into the D3 deleted flagellin which allow proper orientation of the GFP-binding region (indicated by a dotted surface) of the aGFP nanobody. Molecular modelling was done by the UCSF Chimera program (1).

Reference

1. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C. & Ferrin, T. E. UCSF Chimera – a visualization system for exploratory research and analysis. *J. Comput. Chem.* **13**, 1605-1612 (2004).