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Supporting information for article:

Low-resolution structures of modular nanotransporters shed light on their functional activity

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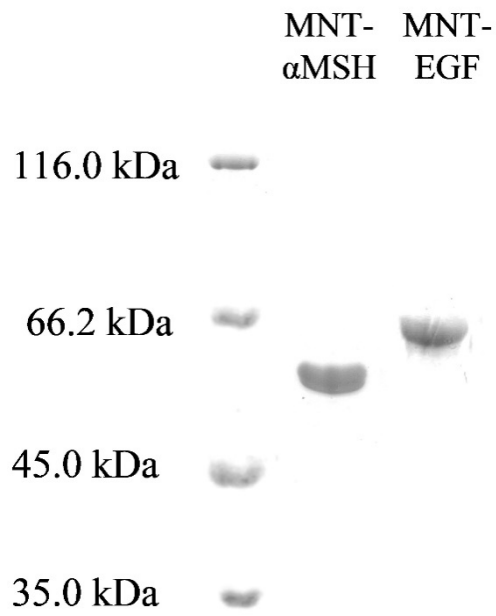

Figure S1 Laemmli SDS-PAGE of MNT-αMSH and MNT-EGF in 12.5% gel. Expected molecular weight of MNT-αMSH and MNT-EGF are 70.4 and 76.4 kDa, respectively.

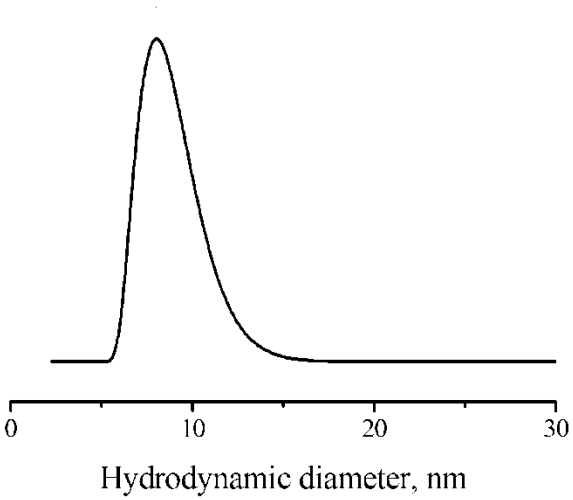

Figure S2 Particle size distribution for a MNT-αMSH sample (μM) in PBS as measured by dynamic light scattering; the mean hydrodynamic diameter of the modular nanotransporter was 8.3 ± 0.6 nm.

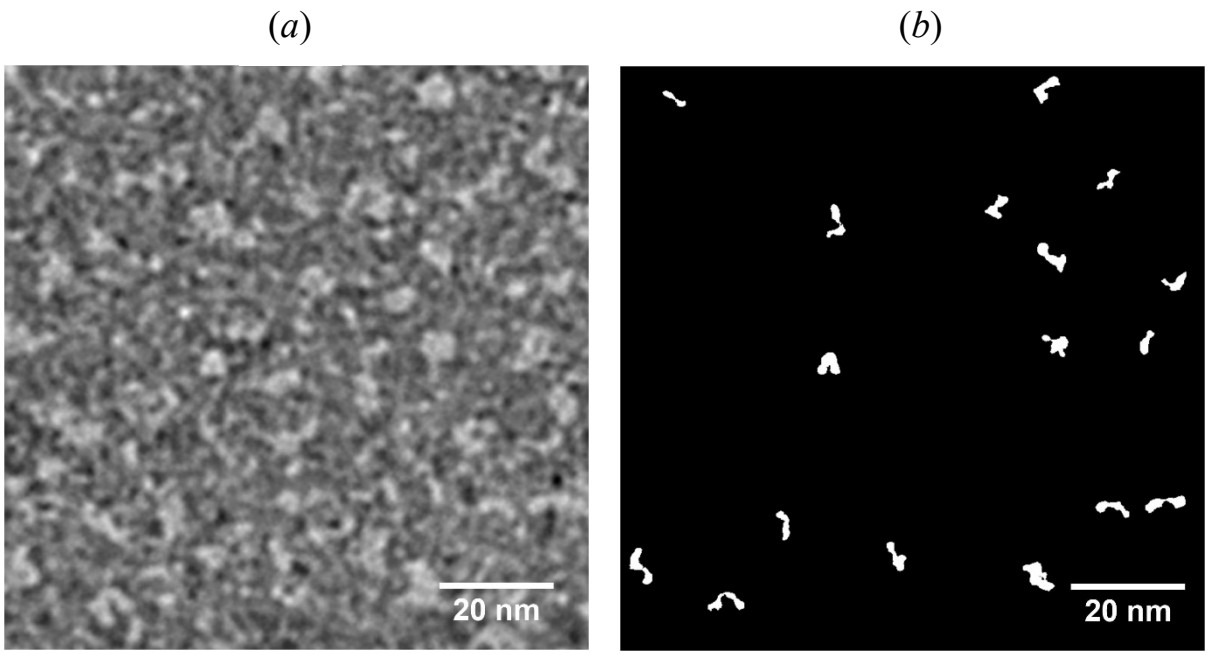

Figure S3 **(*a*)** Example of a TEM image of MNT-EGF particles after Gaussian filtering. **(*b*)** MNT-EGF particles have been marked with an automatic mask.

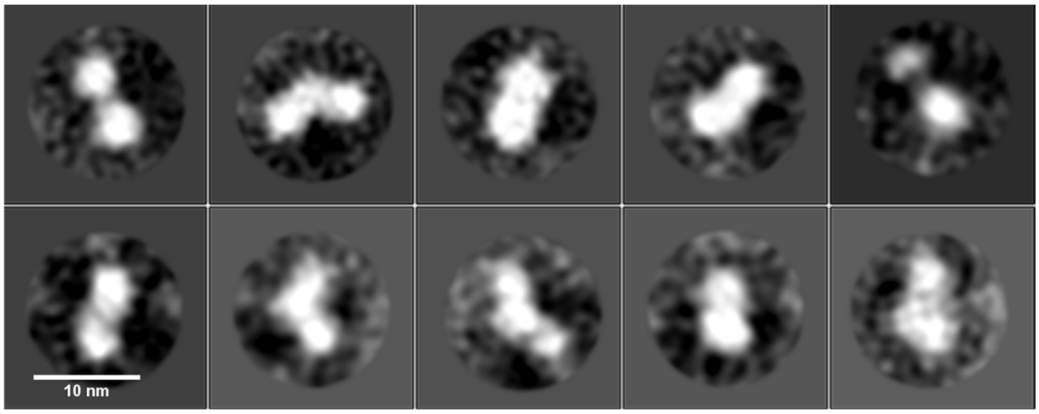

Figure S4 Two-dimensional classes of images obtained by TEM for MNT-αMSH molecules.

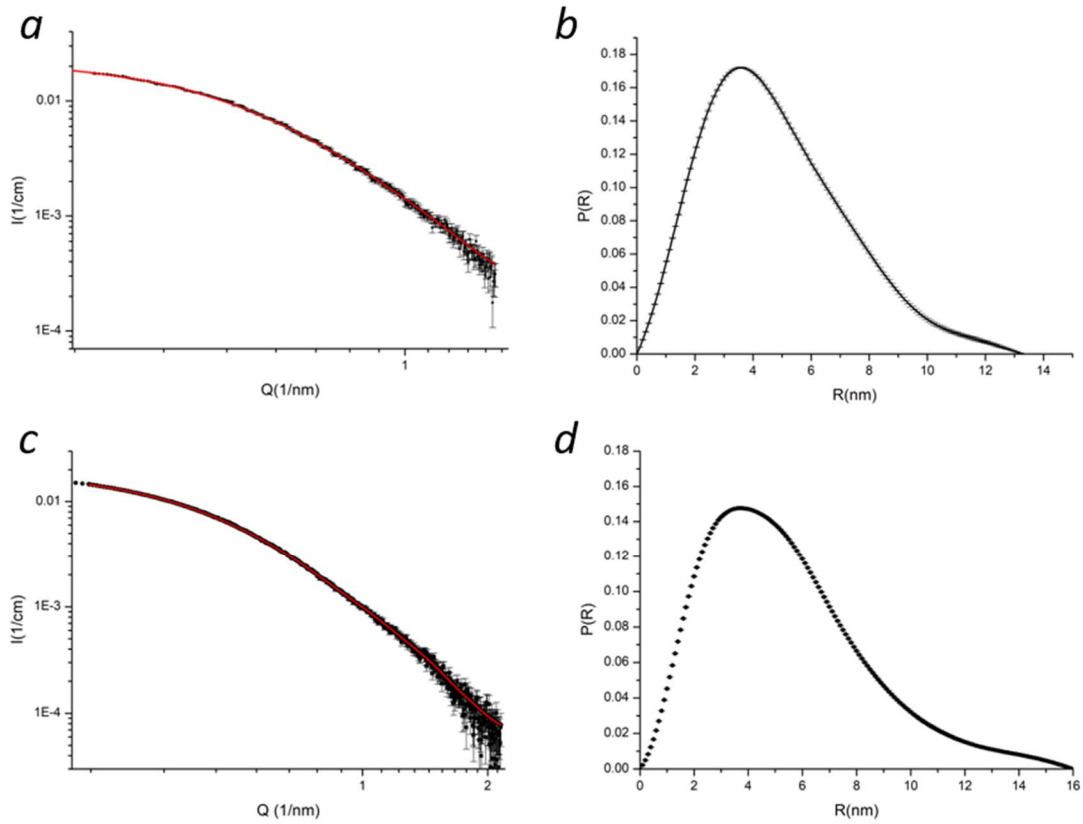

Figure S5 *a* – SAXS curves I(Q) obtained from merging frames that correspond the MNT-αMSH molecules. Black dots show experimental data for MNT-αMSH in PBS (pH 8). Red line shows fit by GNOM (program from ATSAS software package) that was used for 3D low-resolution structure recovering. *b* – Pair-distribution functions for MNT-αMSH in PBS (pH 8). *c* – SAXS curves I(Q) obtained from merging frames that correspond the MNT-EGF molecules. Black dots show experimental data for MNT-EGF in PBS (pH 8). Red line shows fit by GNOM (program from ATSAS software package) that was used for 3D low-resolution structure recovering. *d* – Pair-distribution functions for MNT-EGF in PBS (pH 8).

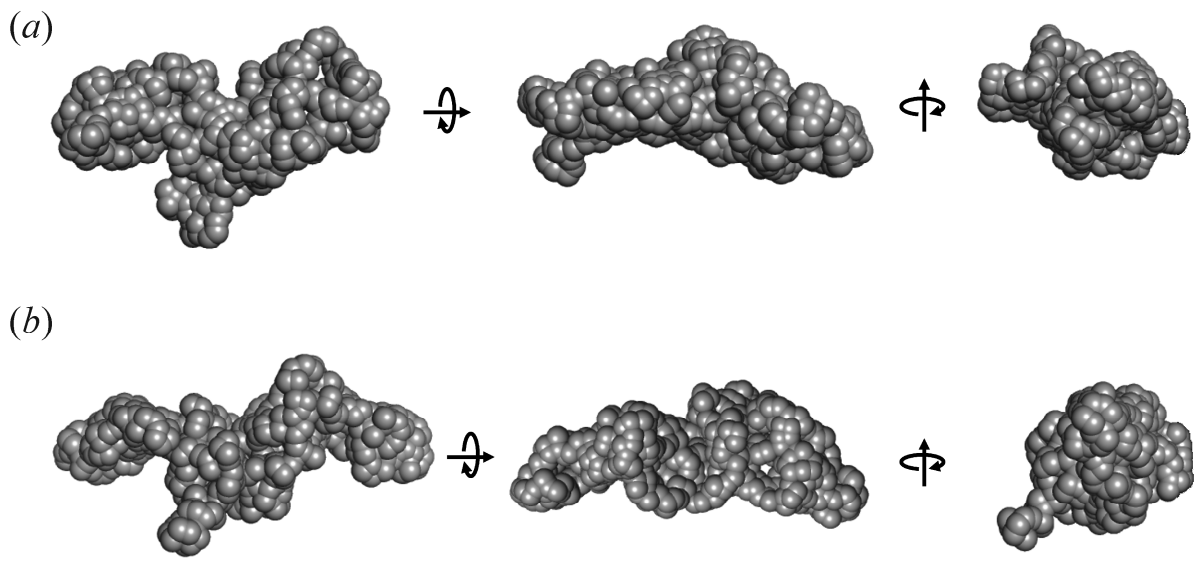

Figure S6 *a* – 3D *ab-initio* low-resolution structures of MNT-αMSH in PBS (pH 8). *b* – 3D *ab-initio* low-resolution structures of MNT-EGF in PBS (pH 8).

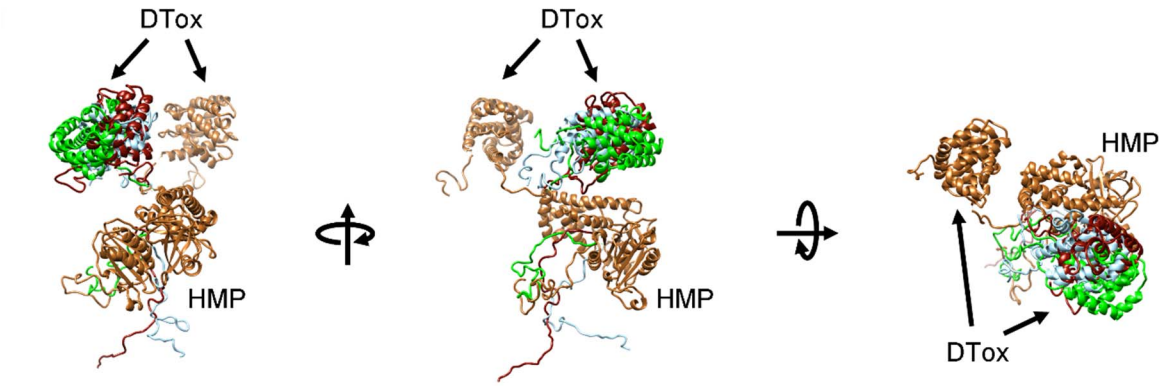

Figure S7 Different views of the ensemble of MNT-αMSH models obtained by EOM program. HMP domain is fixed and DTox domain is free in the input data.

Table S1 SAXS experimental details and data evaluation summary.

***(a)* Sample details**		
	MNT-αMSH	MNT-EGF
Description of sequence	The first part of the amino acid sequence of both entries encompasses sequences homologous to Diphtheria toxin (Uniprot ID P00587) followed by Flavohemoprotein (Uniprot ID P24232). For MNT–MSH, the C-terminal region (CTR) has a sequence homology to the Large T antigen from Simian virus 40 (SV40) (Uniprot ID P03070). For MNT–EGF, the CTR has a sequence homology to Pro-epidermal growth factor (Uniprot ID P01133).	
Extinction coefficient ε (A280, 0.1% cm^{-1})	1.157	1.084
Partial specific volume $\bar{v}$ ($cm^3\ g^{-1}$)	0.728679	0.7286749
Mean solute and solvent scattering length densities (10^{-6} $Å^{-2}$)	12.481, 9.465	12.477, 9.465
Mean scattering contrast $\Delta\bar{\rho}$ (10^{-6} $Å^{-2}$)	3.016	3.012
Molecular mass *M* from chemical composition (Da)	70435.36	76309.76
For SEC-SAS, loading concentration (mg ml^{-1}), injection volume (µl), flow rate (ml min^{-1})	4.2, 500, 0.75	4.2, 500, 0.75
Solvent composition – all samples	10 mM phosphate buffer saline, 150 mM NaCl, pH 8.0	
(*b*) SAS data collection parameters		
Instrument	ESRF BM29	
Wavelength (Å)	0.9918	
Beam geometry (size, sample-to-detector distance)	700 × 700 $µm^2$, 2.864 m	
q-measurement range ($Å^{-1}$)	0.008 – 0.45	
Absolute scaling method	Comparison with scattering from pure H_2O	
Basis for normalization to constant counts	To transmitted intensity by beam stop counter	
Exposure time, number of exposures	1 frame/sec 101 frames	1 frame/sec 10 frames
Sample configuration including path length and flow rate	SEC–SAXS experiments were performed with the column Superdex 200 (S200 GL 10/300). Samples were exposed to X-rays while flowing through the 1.8 mm-diameter quartz capillary at a flow-rate of 0.75 ml/sec.	
Sample temperature (ºC)	20	
(*c*) Software employed for SAS data reduction, analysis and interpretation		
SAS data averaging, reduction to sample−solvent scattering, merging	PRIMUS from ATSAS 2.8.4	
Calculation of ε from sequence	ProtParam: https://web.expasy.org/protparam/	
Calculation of $\bar{v}$ values from chemical composition	Peptide Property Calculator: http://biotools.nubic.northwestern.edu/proteincalc.html	
Calculation of $\bar{\rho}$ values from chemical composition	SLD calculator web: https://sld-calculator.appspot.com/	
Guinier, *P*(*r*), Porod volume (V_P)	PRIMUSqt from ATSAS 2.8.4	
Shape/bead modelling	Gasbor from ATSAS 3.0.1	
Atomic structure modelling (rigid body, ensemble)	EOM from ATSAS 3.0.1 (only for MNT–MSH)	
Molecular graphics	PyMOL v 1.9.x, UCSF Chimera 1.14	
(*d*) Structural parameters		
Guinier analysis	MNT-αMSH	MNT-EGF
R_g (Å)	37.2 ± 0.9	41.9 ± 0.5
q-range ($Å^{-1}$) (qRg maximum)	0.02436 – 0.03001 (1.12)	0.02201 – 0.2718
Fidelity	0.26	0.47
P(*r*) analysis	MNT–MSH	MNT–EGF
R_g (Å)	37.8 ± 0.23	43.0 ± 0.18
d_{max} (Å)	133	160
q-range ($Å^{-1}$)	0.02436 – 0.1515	0.02201 – 0.2150
Total quality estimate (GNOM)	0.641	0.556
Volume (V_P) ($Å^3$)	130610	156020
(*e*) Shape modelling results (Gasbor)		

	MNT-αMSH	MNT-EGF
q-range for fitting	0.02436 – 0.1515	0.02201 – 0.2150
Symmetry/anisotropy assumptions	P1	P1
χ^2 value	0.8873	1.126
P value	0.91501	0.043664
Constant subtraction in optimization	0.0001726	0.000005346
Model volume (Å^3)	10846	11798
Model resolution (from q_{max})	41 Å	29 Å
(*f*) Atomistic modelling		
	MNT-αMSH	MNT-EGF
Method	Ensemble Optimization Method (EOM)	–
q-range for fitting	0.02436 – 0.1515	–
Symmetry assumptions	P1	–
Any measures of model precision		–
χ^2 value	0.739	–
P value	0.999578	–
Constant subtraction in optimization	0.000	–
R_g values (Å), d_{max} values (Å), and weights for multi-state model	35.00 145.43 ~0.14 (1/7) 42.17 132.83 ~0.29 (2/7) 33.73 103.74 ~0.29 (2/7) 33.89 119.74 ~0.29 (2/7)	–
Final ensemble R_g (Å) and d_{max} (Å)	36.37, 122.58	–
(*g*) Data and model deposition IDs		
	MNT-αMSH	MNT-EGF
	SASDJY7	SASDJZ7