Supplementary Information

Structural insights and aggregation propensity of a super-stable MNEI mutant: a new potential building block for protein-based nanostructured materials

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Table S1. RMSD between Cα (Å) of the two chains, A and B, of MNEI and Mut9.

		MNEI		**Mut9**	
		chain A	***chain B***	***chain A***	***chain B***
MNEI	chain A	-			
	chain B	0.299	-		
Mut9	chain A	0.236	0.317	-	
	chain B	0.335	0.311	0.236	-

Table S2. RMSD between Cα (Å) of the two chains, A and B, of MNEI and Mut9 and of the crystallographic structures of MNEI and mutants reported in the literature.

PDB code	Point mutations	Chain	MNEI		Mut9	
			chain A	*chain B*	*chain A*	*chain B*
2O9U	//	X	0.579	0.505	0.526	0.546
3PXM	V37A	A	0.332	0.47	0.452	0.557
		B	0.587	0.446	0.682	0.599
3PYJ	G16A	A	0.433	0.621	0.461	0.567
3Q2P	G16A; V38A	A	0.307	0.399	0.375	0.38
		B	0.34	0.357	0.41	0.386
		C	0.341	0.504	0.35	0.379
		D	0.328	0.373	0.335	0.389
5LC6	Q28K; C41S; Y65R	A	0.459	0.671	0.423	0.525
		B	0.327	0.365	0.271	0.361
5LC7	E23Q; Q28K; C41S; Y65R	A	0.288	0.234	0.244	0.231
		B	0.201	0.301	0.229	0.291
5O7K	Y65R	A	0.423	0.6	0.445	0.569
		B	0.361	0.341	0.316	0.385
5O7L	Y65R	A	0.498	0.723	0.48	0.606
		B	0.37	0.382	0.33	0.416
5O7Q	Y65R	A	0.342	0.492	0.376	0.461
		B	0.457	0.468	0.448	0.515
5O7R	Y65R	A	0.323	0.314	0.371	0.349
		B	0.314	0.421	0.322	0.388
5O7S	Y65R	A	0.345	0.35	0.365	0.387
		B	0.327	0.465	0.424	0.442
5Z1P	E3N; E24A	A	0.354	0.341	0.347	0.364
		B	0.324	0.432	0.352	0.372
		C	0.358	0.521	0.404	0.43

		D	0.349	0.327	0.329	0.355

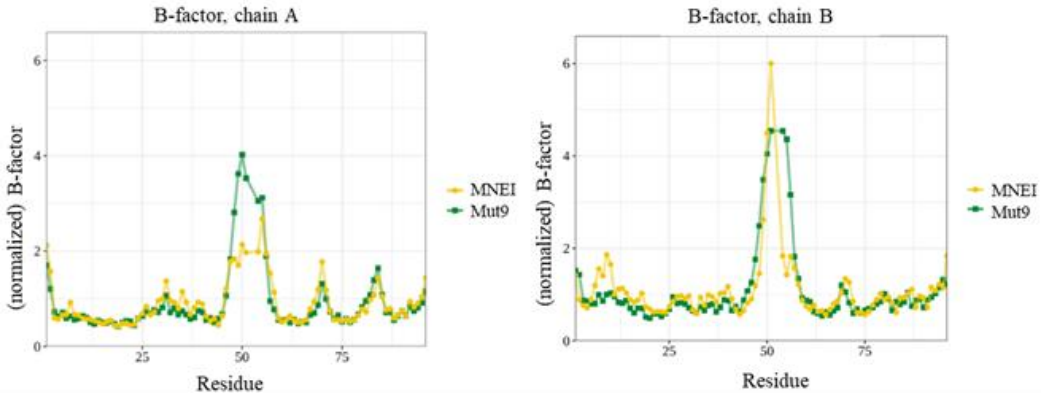


Fig. S1. Graphic representations of B-relative trend as a function of residue in chains A and B of MNEI and Mut9.

Table S3. Analysis of the structural features of MNEI and Mut9.

Protein	**MNEI**		**Mut9**	
Chain	A	B	A	B
Number of hydrogen bonds (Main chain)	57	60	58	54
Number of hydrogen bonds (Side chain)	26	20	19	19
Number of salt bridges	0	0	0	0
Accessible solvent area (Total ASA) (Å^2)	6086.4	5976.9	6064.4	6068.9
Non-polar surface (Å^2)	3496.7	3402.4	3417.2	3416.2
Polar surface (Å^2)	1127.6	1116.5	1144.5	1095.7
Charged surface (Å^2)	1462.1	1458.0	1502.8	1557.0
Surface area between the two molecules of the a.u. (Å^2)	865.748		903.476	
Volume (Å^3)	13116.2	13080.6	13178.5	13329.6
Compactness	2.2629	2.2262	2.2476	2.2323

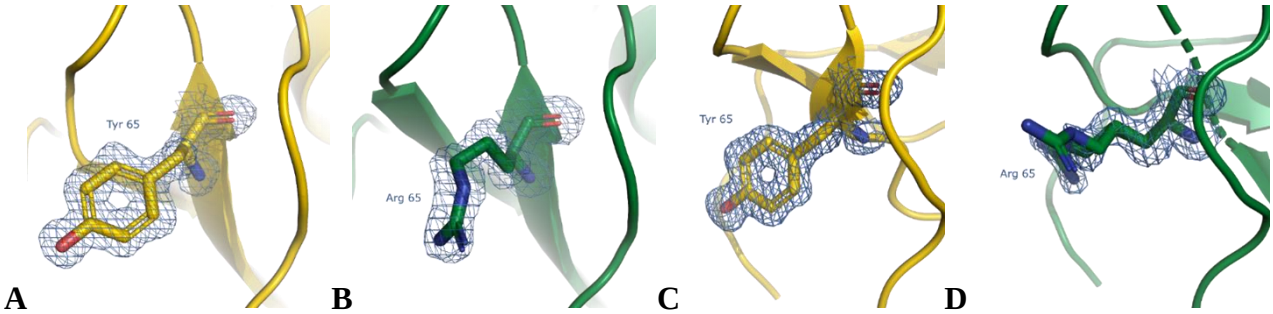


Fig. S2. 2Fo-Fc electron density map of residue 65 in MNEI (yellow) and Mut9 (green). Chains A are reported in panels A and B, chains B in panel C and D. Electron density is contoured at 1σ.

Table S4. Distances (Å) between residues 63 and 94 in both molecules of the asymmetric unit of MNEI and Mut9. The distances have been calculated also for Y65R structures by Pica et al. (ref. 20 in the main text) for comparison.

PDB code	**Mutation**	**pH**	**Distance (Å)**			
			Y63, OH - P94, O (chain A)	***Y63, OH - X - P94, O (chain A)***	***Y63, OH - P94, O (chain B)***	***Y63, OH - X - P94, O (chain B)***
MNEI	//	4.6	5.28	3.56---H_2O---2.85	4.45	2.60---H_2O---2.65
Mut9	E23A; C41A; Y65R; S76Y	4.6	5.25	3.29---H_2O---2.70	4.85	2.76---H_2O---2.80
5O7L	Y65R	4.6	6.53	2.66---SO_4---5.74	6.06	2.79--- SO_4---4.96
1MOL (WT)	//	7.4	5.15	-	5.59	3.72---H_2O---2.70
2O9U* (WT)	//	5.6	4.04	2.65---H_2O---2.67	4.76	2.65---H_2O---2.80
5O7K	Y65R	2	5.83	2.77---H_2O---5.17	5.73	2.49---SO_4---5.24
5O7Q	Y65R	5.5	5.94	4.14---H_2O---2.44	5.73	4.10---H_2O---2.78
5O7R	Y65R	6.5	5.69	3.87---H_2O---2.58	5.67	4.12---H_2O---2.41
5O7S	Y65R	8.3	5.4	2.73---H_2O---3.09	5.57	3.52---H_2O---2.53

*The 2O9U protein has a single chain; The values in this case are relative to an alternative conformation.

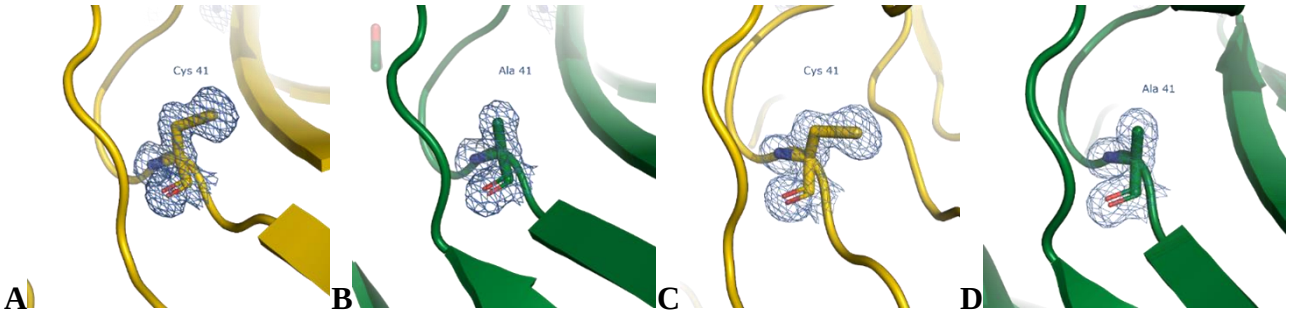


Fig. S3. 2Fo-Fc electron density map of residue 41 in MNEI (yellow) and Mut9 (green). Chains A are reported in panels A and B, chains B in panel C and D. Electron density is contoured at 1σ.

Table S5. Distances (Å) between residues 41 and 62 in both molecules of the asymmetric unit of MNEI and Mut9.

	Distance (Å)			
	Residue 41, CA - L62, CA (chain A)	***Residue 41, CB - L62, CD (chain A)***	***Residue 41, CA - L62, CA (chain B)***	***Residue 41, CB - L62, CD (chain B)***
MNEI	5.23	5.04	4.98	5.01
Mut9	5.11	3.66	5.01	3.60

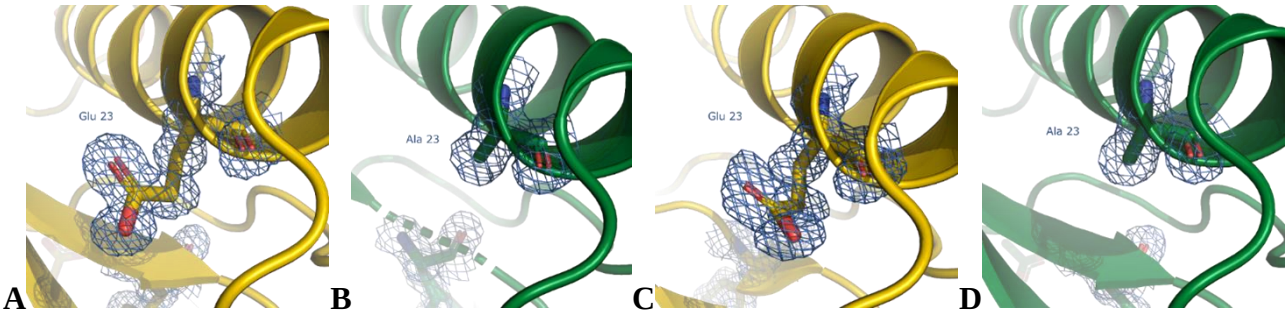


Fig. S4. 2Fo-Fc electron density map of residue 23 in MNEI (yellow) and Mut9 (green). Chains A are reported in panels A and B, chains B in panel C and D. Electron density is contoured at 1σ.

Table S6. Distances (Å) between residues 23 and 89 in both molecules of the asymmetric unit of MNEI and Mut9.

	Distance (Å)	
	Residue 23, CA - F89, CA (chain A)	***Residue 23, CA - F89, CA (chain B)***
MNEI	7.12	6.95
Mut9	6.61	6.63

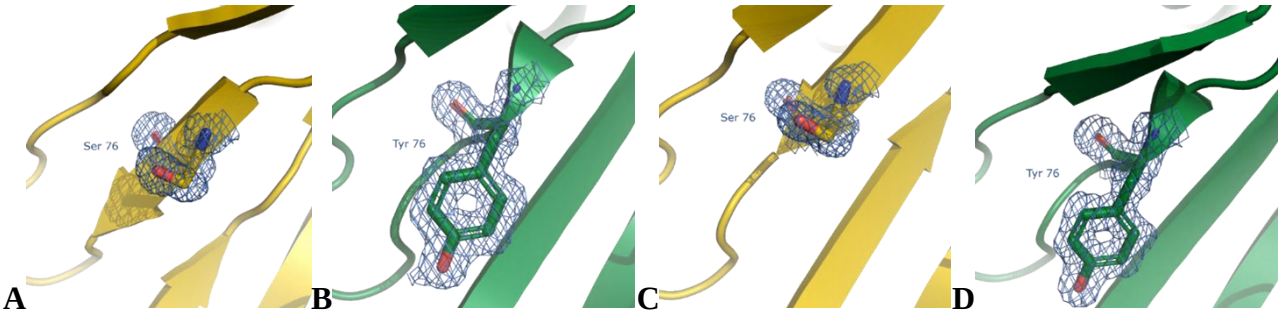


Fig. S5. 2Fo-Fc electron density map of residue 76 in MNEI (yellow) and Mut9 (green). Chains A are reported in panels A and B, chains B in panel C and D. Electron density is contoured at 1σ.

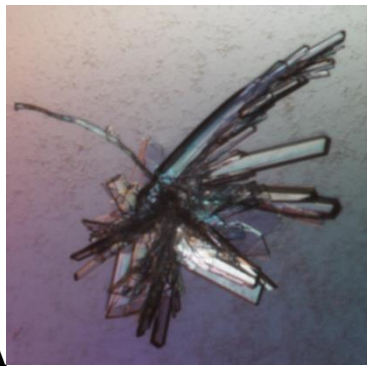
A  **B** 

Fig. S6. Crystals of MNEI (A) and Mut9 (B).

Table S7. Data collection and refinement statistics for MNEI and Mut9.

Data collection statistics		
Protein	MNEI	Mut9
PDB code	8Q0S	8Q0R
Space group	P1	P1
Unit cell parameters **a, b, c (Å)** **α, β, γ (°)**	 31.65, 38.97, 44.13 106.00, 109.52, 103.36	 31.72, 39.35, 43.89 105.35, 109.33, 103.23
Molecules in the asymmetric unit	2	2
Observed reflections	179068 (8015)	91391 (3387)
Unique reflections	52760 (2446)	27470 (1225)
Resolution (Å)	38.39 – 1.19 (1.21 - 1.19)	35.58 – 1.50 (1.53 – 1.50)
Completeness (%)	92.7 (85.4)	94.7 (84.6)
Rmerge	0.037 (0.679)	0.043 (0.489)
Rpim	0.024 (0.443)	0.028 (0.360)
Rmeas	0.044 (0.815)	0.052 (0.612)
I/I(σ)	14.2 (2.1)	16.3 (2.3)
Multiplicity	3.4(3.3)	3.3 (2.8)
$CC_{1/2}$	0.999(0.726)	0.999 (0.690)
Refinement statistics		
Resolution (Å)	38.39 - 1.19	35.58 – 1.50
N° reflection (working set)	47541	25065
N° reflection (test set)	2337	1541
N° non-H atoms (refinement)	2103	1906
R-factor/Rfree	0.218/0.256	0.223/0.274
r.m.s.d. from ideality		
r.m.s.d. bonds	0.012	0.010
r.m.s.d. angles (°)	1.768	1.625