Supplemental Information

Supplementary Figure 1 a) Crystals of *E. coli* DHFR-MTX^2-C9

a

b

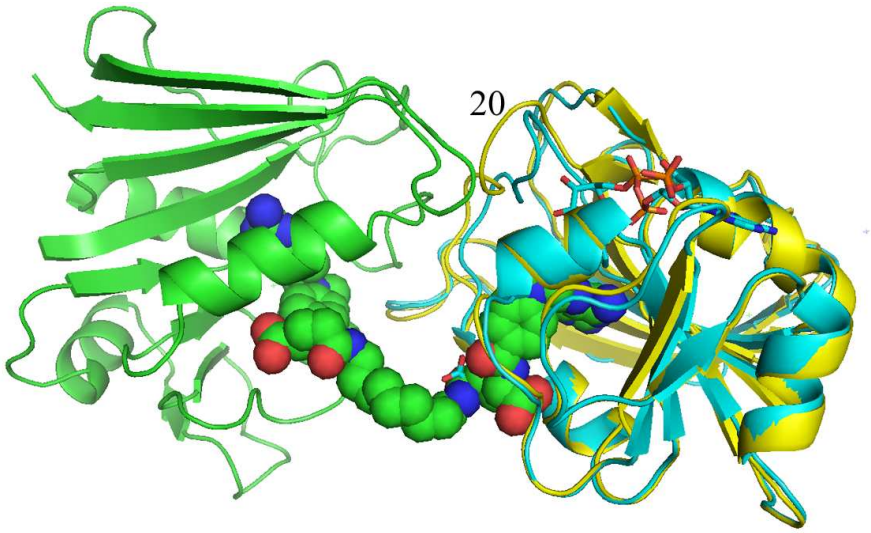
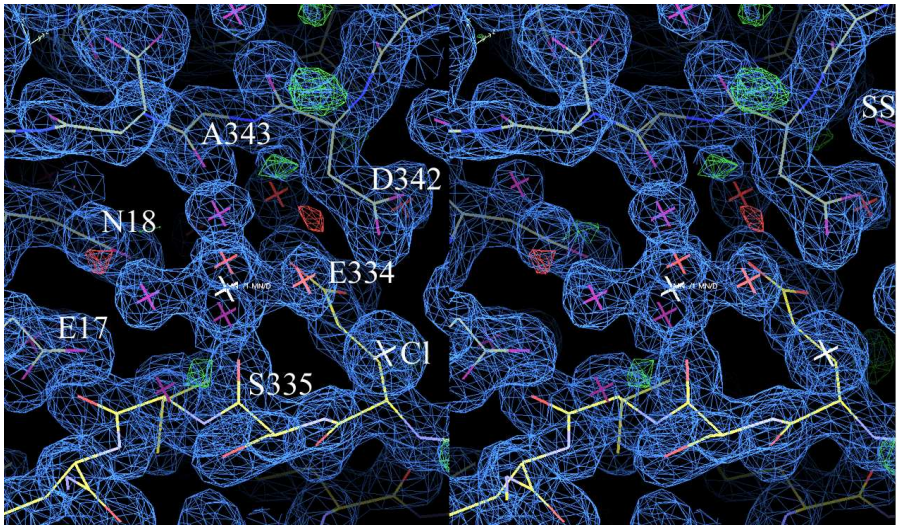
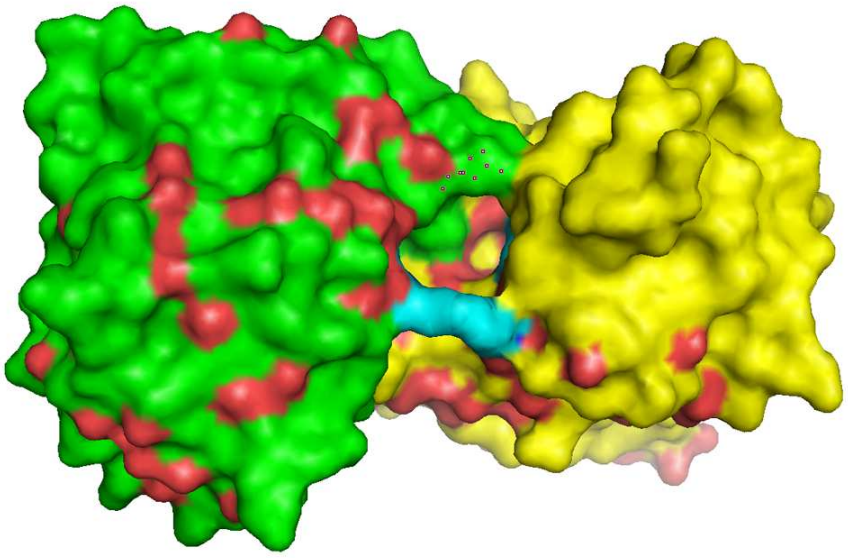


Fig. 1b. Crystal structure of MTX^2-C9 (space filling) binary complex with ecDHFR (green and yellow monomers) compared with ternary complex with ecDHFR-MTX-NADPH (cyan). The major difference between the monomeric ecDHFR-MTX-NADPH (cyan) complex and that of the ecDHFR MTX^2-C9 binary complex (green and yellow) is in the position of loop 20 (labeled) near the binding site of NADPH (stick figure).

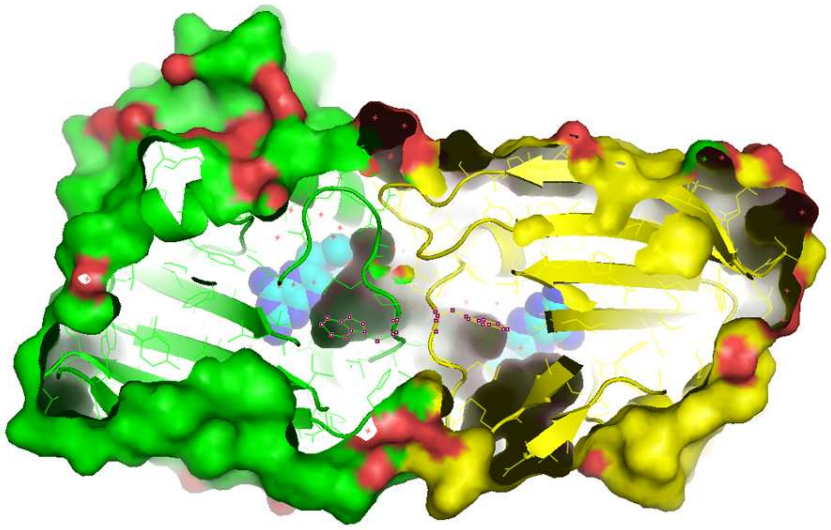


c. Stereo view of the Mn binding site in binary complex *E. coli* DHFR-MTX^2-C9.

The 2Fo-Fc difference density is contoured at 1σ. Red X is water and purple X is water from symmetry-related position. The white X is chloride.



d. *E. coli* DHFR-MTX^2-C9 with van der Waals surface with monomer 1 (green) and monomer 2 (yellow) and MTX^2-C9 (cyan). The red dots show the position of Trp146 that form part of the dimer interface.



e. Clipped view of the van der Waals surface of *E. coli* DHFR-MTX^2-C9 highlighting the dimer interface between monomer 1 (green) and monomer 2 (yellow) with MTX^2-C9 (cyan). Trp22 in both domains is shown in dotted outline.

Supplementary Table 1. Data collection and refinement statistics for *E. coli* DHFR and MTX-C9 dimer.

Data collection

PDB accession #	3OCH
Space group	P61
Cell dimensions (Å)	a = b = 91.71, c = 73.13
Beamline	CHESS A1
Resolution (Å)	1.80
Wavelength (Å)	1.00
R_{sym} (%) [a,b]	0.094
Completeness (%) [a]	98.8

Observed reflections	32398
Unique reflections	30751
I/□(I)	2.9
Multiplicity [a]	7.7

Refinement and model quality

Resolution range (Å)	23.20 – 1.79
No. of reflections	30751
R-factor [c]	0.20
R_{free}-factor [d]	0.24
Total protein atoms	2744
Total water atoms	129
Average B-factor ($Å^2$)	19.0

Rms deviation from ideal

Bond lengths (Å)	0.024
Bond angles (^{o})	2.22
Luzzati Plot error	0.207

Ramachandran plot

Residues in most favored regions (%)	97.8
Residues in additional allowed regions (%)	1.9
Residues in generously allowed regions (%)	0.3
Residues in disallowed regions (%)	0.0

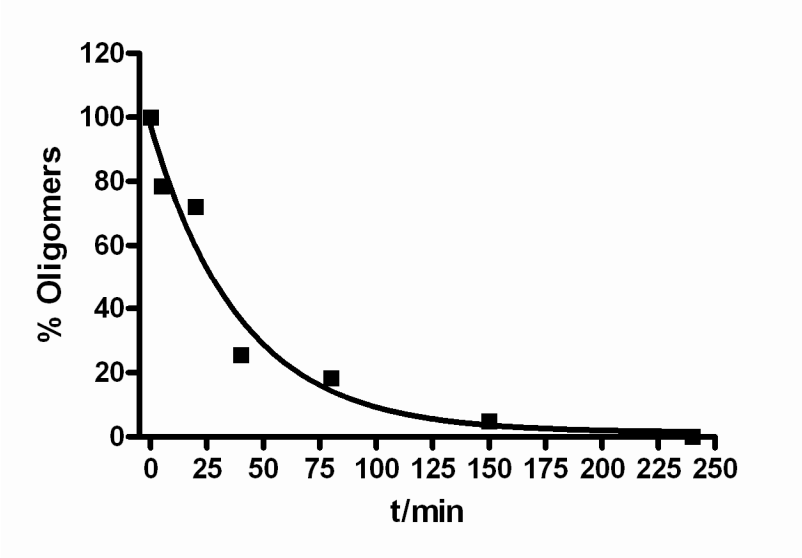
[a] The values in parentheses refer to data in the highest resolution shell.

[b] $R_{sym} = \Sigma_h\Sigma_i|I_{h,i} - \langle I_h\rangle| / \Sigma_h\Sigma_i|I_{h,i}|$, where $\langle I_h\rangle$ is the mean intensity of a set of equivalent reflections.

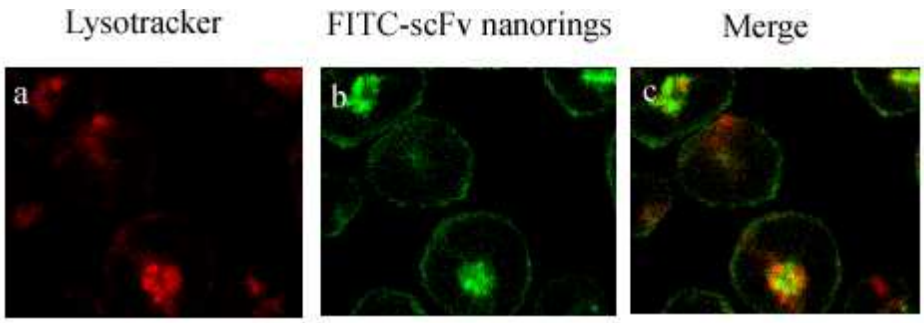
[c] R-factor = $\Sigma|F_{obs} - F_{calc}| / \Sigma F_{obs}$, where F_{obs} and F_{calc} are observed and calculated structure factor amplitudes.

[d] R_{free}-factor was calculated for R-factor for a random 5% subset of all reflections.

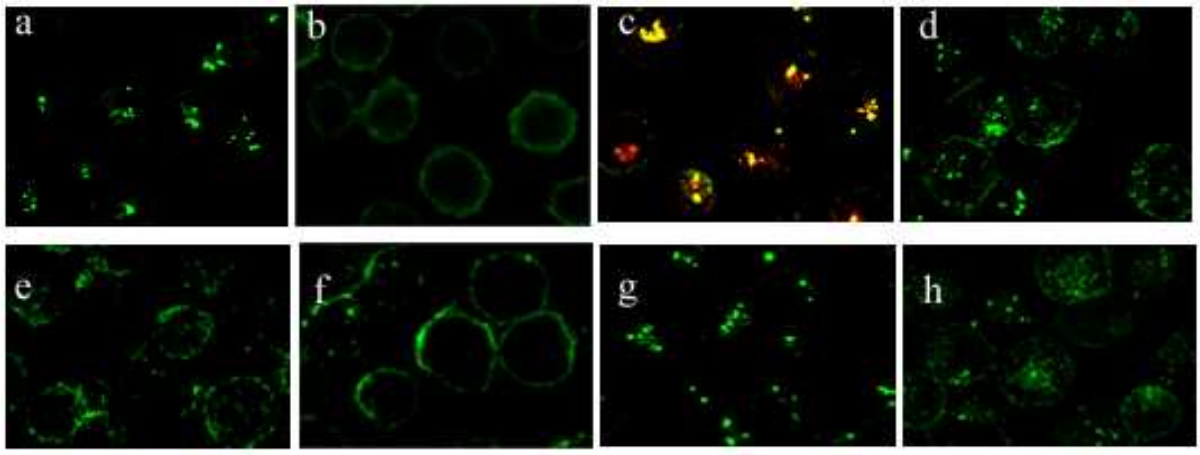
Supplementary Figure 2 Time course study of disassembly of octavalent CSANs by incubation with excess amount of methotrexate. %oligomers: Percentage of oligomers left in the disassembly reaction after incubating with methotrexate at different time points.



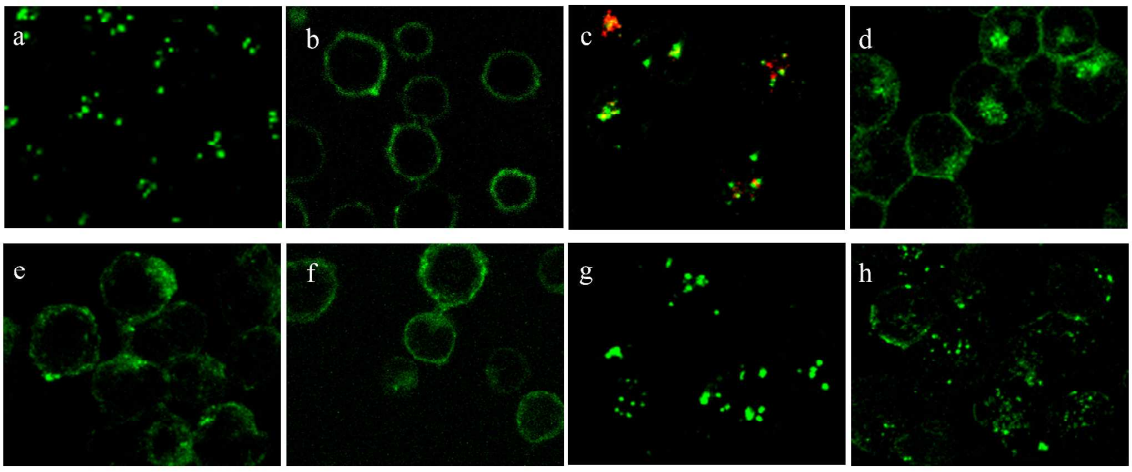
Supplementary Figure 3 Confocal images of HPB-MLT T leukemia cells incubated with FITC-labeled octavalent anti-CD3 CSANs (b) for 30 mins at 37ºC. Image (a) showed lysosomal compartments of the cells. Superposition of red channel and green channel were shown in image c.



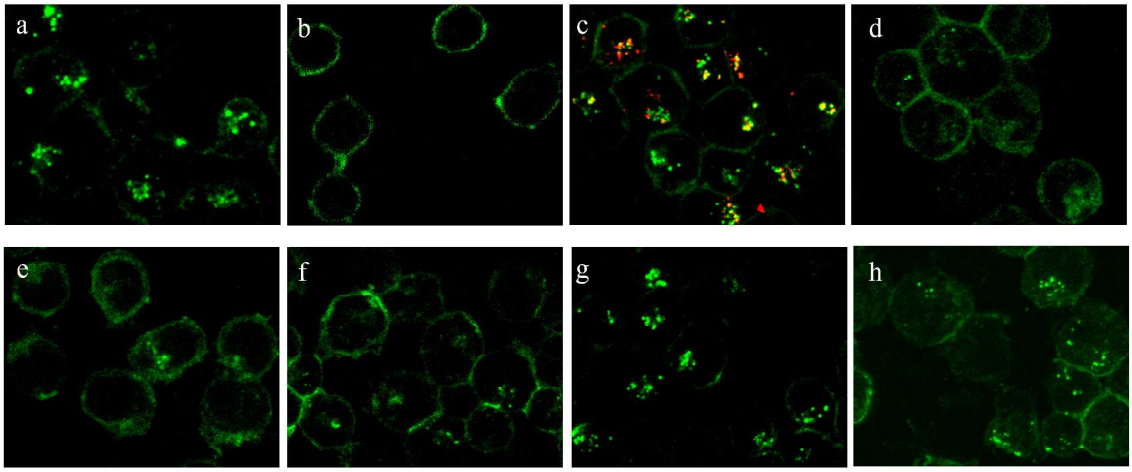
Supplementary Figure 4 Internalization studies of FITC labeled UCHT-1 (green) to HPB-MLT T-leukemia cells. Incubation at (a) 37 ºC or (b) 4 ºC. (c) Colocalization with Alexa Fluor 594-labeled transferrin (red) Colocalization with Alexa Fluor 594-labeled transferrin (red) after incubation at 37 ºC. Incubation in the presence of clathrin-dependent endocytosis inhibitor chlorpromazine (d), actin polymerization inhibitor cytochalasin D (e), protein tyrosine kinase inhibitor genistein (f), caveolin-dependent endocytosis inhibitor nystatin (g), or cholesterol depletion agent methyl-β-cyclodextrin (h) at 37 ºC. All incubations were for 30 min.



Supplementary Figure 5 Internalization studies of FITC labeled 1DDantiCD3 scFv monomer (green) to HPB-MLT T-leukemia cells. a) Incubation at 37 ºC or (b) 4 ºC. (c) Colocalization with Alexa Fluor 594-labeled transferrin (red) after incubation at 37 ºC. Incubation in the presence of clathrin-dependent endocytosis inhibitor chlorpromazine (d), actin polymerization inhibitor cytochalasin D (e), protein tyrosine kinase inhibitor genistein (f), caveolin-dependent endocytosis inhibitor nystatin (g), or cholesterol depletion agent methyl-β-cyclodextrin (h) at 37 ºC. All incubations were for 30 min.



Supplementary Figure 6 Internalization studies of FITC labeled divalent CSANs (13DDantiCD3 scFv dimer, green) to HPB-MLT T-leukemia cells. a) Incubation at 37 ºC or (b) 4 ºC. (c) Colocalization with Alexa Fluor 594-labeled transferrin (red) after incubation at 37 ºC. Incubation in the presence of clathrin-dependent endocytosis inhibitor chlorpromazine (d), actin polymerization inhibitor cytochalasin D (e), protein tyrosine kinase inhibitor genistein (f), caveolin-dependent endocytosis inhibitor nystatin (g), or cholesterol depletion agent methyl-β-cyclodextrin (h) at 37 ºC. All incubations were for 30 min.



Supplementary Figure 7 FACS raw data for the Effects of IL-2R (CD25) expression level on CD4+ cells by triggering with a panel of anti-CD3. PBMCs were incubated with anti-CD3 for 20 hours, and then stained with mAbs of CD4 and CD25. A representative experiment of 3 is shown in a-f. (a) Unstimulated PBMCs, (b) monomeric 1DDantiCD3 stimulated PBMCs, (c) dimeric 13DDantiCD3 stimulated PBMCs, (d) octameric 1DDantiCD3 scFv nanorings stimulated PBMCs, (e) UCHT-1 F(ab')2 stimulated PBMCs, and (f) UCHT-1 stimulated PBMCs.