Supplementary Material

Identifying Binding Hot Spots on Protein Surfaces by Mixed-Solvent MD: HIV-1 Protease as a Test Case

Peter M.-U. Ung,[1,2] Phani Ghanakota,[1] Sarah E. Graham,[3] Katrina W. Lexa,[1] Heather A. Carlson[1,3]*

[1] Department of Medicinal Chemistry, College of Pharmacy, 428 Church St., University of Michigan, Ann Arbor, MI 48109-1065, United States

[2] Present address: Department of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

[3] Department of Biophysics, College of LSA, 930 N. University St., University of Michigan, Ann Arbor, MI 48109-1055, United States

SUPPORTING TABLES

Table S1. Ratio of Organic Solvent and Water in 50% w/w Organic-Aqueous Solutions

	Acetonitrile[1] : water[2]	Isopropanol[3] : water	Pyrimidine[4] : water
Weight : Weight	50 : 50	50 : 50	50 : 50
Mol : Mol	1.220 : 2.775	0.832 : 2.775	0.624 : 2.775
Molecule : Molecule	2,766 : 6,113	829 : 2,779	622 : 2,751

[1] Acetonitrile (ACN): 41.05 g/mol; [2] Water: 18.02 g/mol
[3] Isopropanol (IPA): 60.10 g/mol; [4] Pyrimidine (1P3): 80.09 g/mol

Table S2. Normalized eigenvalues of the first five ED eigenvectors of pure-water MD and 50% w/w MixMD

	Normalized Eigenvalue (Cumulative Percentage)			
Eigenvector	Pure Water	ACN 50%	IPA50%	1P3 50%
1	0.54 (54%)	0.29 (29%)	0.39 (39%)	0.54 (54%)
2	0.14 (68%)	0.18 (47%)	0.21 (60%)	0.14 (68%)
3	0.08 (75%)	0.14 (60%)	0.11 (71%)	0.09 (78%)
4	0.06 (81%)	0.08 (68%)	0.06 (77%)	0.04 (82%)
5	0.04 (86%)	0.07 (75%)	0.05 (82%)	0.04 (86%)

Table S3. Ratio of organic solvent and water molecule in 5% v/v organic-water solutions

	ACN : water[2]	IPA : water	1P3[4] : water
Volume : Volume	5 : 95	5 : 95	5 : 95
Weight : Weight	3.10 : 95	5.08 : 95	3.93 : 95
Mol : mol	1.0 : 55.5	1.0 : 83.2	1.0 : 80.7
Molecule : Molecule	276 : 15,315	184 : 15,308	190 : 15,333

[1] ACN: 41.05 g/mol; [2] Water: 18.02 g/mol
[3] IPA: 60.10 g/mol; [4] 1P3: 80.09 g/mol

SUPPORTING FIGURES

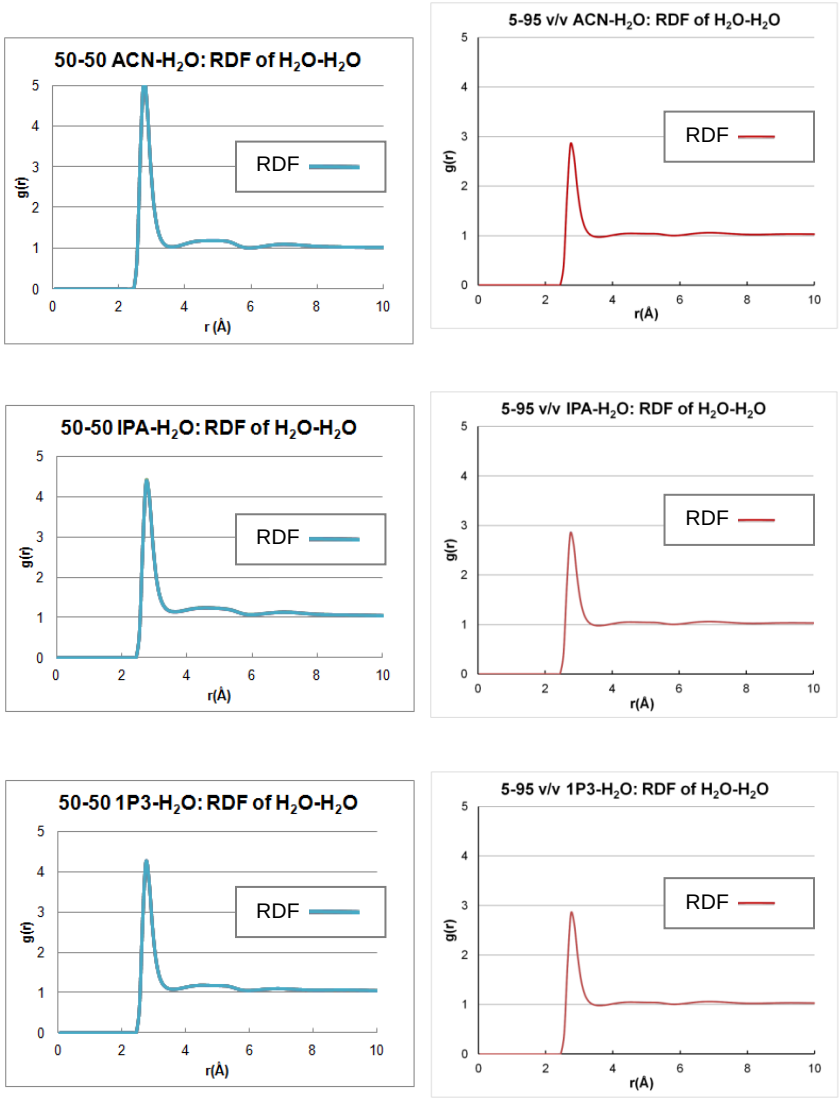

Figure S1. Different solvents have differently shaped radial distribution functions, so to put all the simulations into a common frame of reference, the RDFs of water in each mixed-solvent system are given. If water is evenly mixed, the partner solvent of ACN, IPA, or 1P3 must also be evenly mixed. **On the left in blue,** the RDFs are given for the simulations of semi-open HIV-1p in 50% w/w solvent environments. **On the right in red,** the RDFs are given for the simulations of semi-open HIV-1p in 5% v/v solvent environments. The even mixing of all solvents is confirmed by finding the same, standard RDFs for TIP3P water that converge to 1.0 at ~8 Å. .

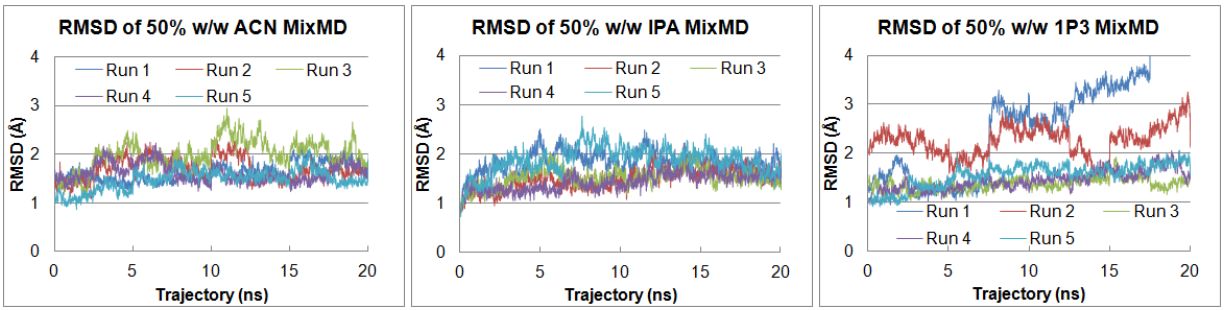

Figure S2. RMSD of the core of the HIV-1p dimer in 50% w/w MixMD. The trajectories are stable throughout the 20-ns simulation, with the exception of one opening event in 1P3. A similar opening event has been observed in pure-water simulations. [ref 34 in manuscript]

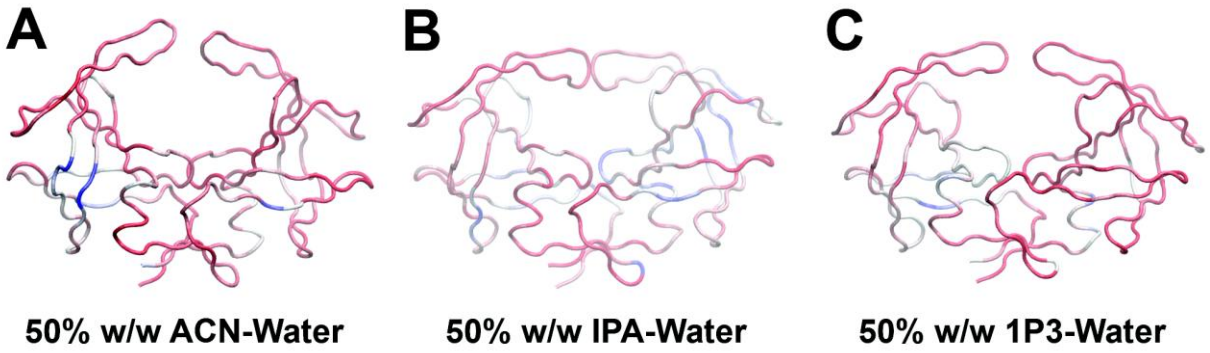

Figure S3. Comparison of the 1st ED eigenvectors of apo HIV-1p in water and in 50% w/w probe-water solution MixMD. Red indicates correlated vectors with positive dot-products. Blue indicates anti-correlated vectors with negative dot-products. White indicates the vectors have no correlation. (A) 1st ED eigenvectors from ACN-water MixMD and from MD have a global similarity factor of 0.734, (B) while IPA-water MixMD and MD is 0.556, and (C) 1P3-water MixMD and MD is 0.760. The illustrated HIV-1p is the averaged structure of 5 independent trajectories of MixMD.